Conservation and Foraging Ecology of Bumble Bees in Urban Environments

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ABSTRACT

The decline of British bumble bees has been attributed to the loss of their habitat through the intensification of agricultural practices. In the quest for information of use to bumble bee conservation the potential of our flower-rich cities has been overlooked. The overall aim of this study was to determine the status and foraging requirements of bumble bees in the urban environment provided by the city of London, U.K.

My principal findings are as follows. Six common species and three rare species were identified. The greatest diversity of *Bombus* species was found in the east of London. Garden and wasteland habitats attracted the greatest abundance of workers and diversity of *Bombus* species. The distribution of *Bombus humilis* (a Biodiversity Action Plan Species) was found to follow the strip of derelict Thames-side industrial land on the eastern side of London as far west as the Millennium Dome, and the River Lea as far north as the Walthamstow Marshes. The phenology of *B. humilis* in London was established. The majority of all observations of foraging *B. humilis* were on flowers in the Lamiaceae family but species native to the U.K. were not necessarily its favoured forage. In a field experiment, the removal of potential competitors significantly increased the time that *B. humilis* workers spent foraging at patches of flowers. Microsatellite analysis was successfully employed to test three hypotheses concerning the movement patterns of foraging *Bombus* workers at three spatial scales. Neither *B. terrestris* nor *B. pascuorum* workers were found to forage with their nest-mates on patches of flowers. Mean numbers of 96 and 66 colonies of *B. terrestris* and *B. pascuorum* respectively were identified foraging on sites with a mean area of 0.8 hectares. No inbreeding and little or no genetic differentiation could be detected in either species across London.

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For Dominic and Eliot who will finally now get the attention that they deserve.

Chapter 1

Introduction

Concern over increasing threats to the world's biodiversity has led to a widespread desire to practice effective conservation and brought to deserved prominence conservation biology as the scientific discipline essential to this goal. Many of the issues relevant to the conservation biology of bumble bees have been addressed in other species of social insect. For example, reserve design (Partridge *et al.*, 1996), habitat management (Matheson *et al.*, 1996), habitat fragmentation (Boswell *et al.*, 1998; Carvalho & Vasconcelos, 1999), and pollution and climate change (Folgarait, 1998), are issues relevant to the conservation biology of terrestrial invertebrates in general. Bumble bees are considered to be essential 'ecological engineers' on account of their role as pollinators (Jones *et al.*, 1994), since they alter the physical environment and affect how resources flow through ecosystems. They also provide irreplaceable 'ecosystem services' to people (Kearns *et al.*, 1998), acting as managed and incidental pollinators of commercial crops, particularly leguminous seed crops and fruit trees (Fussell & Corbet, 1991; Stoddard & Bond, 1987). There are growing concerns over the probable world-wide decline of vertebrate and invertebrate pollinators (e.g. Allen-Wardell *et al.*, 1998; Matheson *et al.*, 1996; Washitani, 1996). Although the symptoms of pollinator scarcity are as serious as reduced fruit and seed set and reduction in yields, there are major gaps in our knowledge of the interactions between invertebrate pollinators and their forage (Kearns & Inouye, 1997). Bumble bees are known to play a significant role in the pollination of wildflowers (Corbet *et al.*, 1991; Kwak *et al.*, 1996;

Osborne *et al.*, 1991), yet the pollination requirements of many species of wild plants in Britain still remain unknown (Corbet *et al.*, 1993).

This study focuses on the non-socially parasitic bumble bees of the genus *Bombus*, of which there are nineteen British species. Of these species, *B. pomorum* has not been seen since 1864 (IBRA/BRC, 1980), *B. cullumanus* has not been seen since 1941 (Falk, 1991) and *B. subterraneus* was declared extinct in 1998 (WWF, 1998). Such is the concern over these extinctions and the declines of other species that five *Bombus* species are included in the U.K. Biodiversity Action Plan Priority List (*B. distinguendus*, *B. humilis*, *B. ruderatus*, *B. subterraneus* and *B. sylvarum*), and three further species are included in English Nature's Species Recovery Programme (*B. monticola*, *B. muscorum*, *B. ruderarius*) (Anonymous, 1995, 1999). Threats to bumble bees and possible causes for their decline fall into two broad categories, natural and man-made. Natural threats include attack from parasitoids and predation. The pressures exerted by parasitoids and parasites on the dynamics of wild populations of bumble bees are difficult to assess. A variety of Dipteran (Conopidae and Sarcophagidae) and Hymenopteran (Braconidae and Mutilidae) parasitoids have been shown to cause a change in behaviour and ultimately the death of their host as part of their life-cycle (reviewed in: Goulson, 2003). Parasites and commensals such as fungi, protozoa and nematodes are frequently observed within bumble bees and their nests with varying effects (Schmid-Hempel, 1998). In the U.K. natural predators include birds such as shrikes, great tits and wagtails, which forage on individual bees, and mammals such as badgers and mink, which excavate and consume entire nests. The Wax moth *Aphomia sociella* is known to be a voracious predator of bumble bee nests (Pouvreau, 1967). The

adult moth lays its eggs in the bumble bee nest. When they hatch, the moth larvae feed on the comb, the larvae and the pupae, thereby destroying the bumble bee nest. Goulson (2003) observed infestation levels of up to 80% in *B. terrestris* nests in gardens in southern England. He also noted that this rate was much higher than the 20% he observed in farmland habitat. It was not the aim of this study to assess predation risks to bumble bees, but all of the predators mentioned above are present within the study area.

Man-made threats to bumble bees include the loss and fragmentation of habitat. These result in the loss of forage diversity and abundance as well as a reduction in the availability of nest and hibernation sites. In addition, the use of broad spectrum agricultural pesticides has been shown to cause mortalities in honey bees and bumble bees (Thompson & Hunt, 1999). The decline of bumble bees observed in the U.K. has been attributed, in the main part, to the loss of their foraging, nesting and over-wintering habitats through the intensification and mechanisation of agricultural practices (Osborne *et al.*, 1991). For example, between 1932 and 1984 the total area of unimproved grassland in Britain decreased by 90% (Fuller, 1987). The shift from hay-making to silage making, which requires nutrient-enriched 'weed'-free grass to be cut early in the summer whilst still green, has led to the decline of traditional wildflower meadows. Similarly, the removal of hedgerows and headlands to increase the size of fields in order to accommodate increasingly large farm machinery has also reduced the availability of perennial herbaceous vegetation (Osborne *et al.*, 1991; Williams, 1982). The decline of floral diversity has been well documented (Corbet *et al.*, 1991; Muir & Muir, 1987; Williams, 1982). Studies in the U.K. and Europe has shown a direct link

between the floral diversity of an area and the abundance of bee species (Bäckman & Tiainen, 2002; Banaszak, 1996; Kells *et al.*, 2001). Flowers supply the nectar and pollen that bumble bees require for their very existence. Therefore the study of forage selection by bumble bees is of the utmost importance in order to guide conservation management strategies. Of the potential threats to bumble bees, outlined above, the loss of forage diversity is a threat that could be overcome. In a wild population of bumble bees a rampant parasitoid would be very difficult to control, but, given the right guidance, the halt and reversal of declining forage availability is an achievable goal.

Williams (1982, 1986) analysed the distribution and decline of British bumble bees by comparing pre-1960 and post-1960 records. He identified the species that had undergone the most notable decline in Britain as those species at the northern limits of their distribution, or 'southern local species'. He also found that the east-central region of England had undergone the most severe decline and named it the 'central impoverished region'. The southern region of England remains the most species-rich for bumble bees in the U.K. As it is situated within this region and displays a rich and diverse flora, London could be providing, or in future provide, a refuge for species declining in the surrounding countryside. Such an urban area consists of a variety of components ranging from totally artificial, built environments, to semi-natural areas. Private gardens and many public spaces are managed to be florally attractive throughout the year and therefore have the potential to sustain foraging bumble bees. The urban floral succession is almost entirely manipulated by man and could therefore be manipulated in favour of a range of pollinators, including bumble bees. There is a large body of work exploring the forage requirements of bumble bees (reviewed in:

Goulson, 2003). The majority of this work has been carried out either in experimental plots (Comba *et al.*, 1999a, 1999b; Corbet *et al.*, 2001), in agricultural environments (Dramstad & Fry, 1995; Svensson *et al.*, 2000), or in exceptional habitats such as Sites of Special Scientific Interest (Carvell, 2001, 2002; Williams, 1988). Although some research has been carried out in suburban areas (Goulson *et al.*, 2000), cities (Saure, 1996), and even in London (Fussell & Corbet, 1993; Goodwin, 1992, 1995), the potential of our flower-rich cities has generally been overlooked in the quest for information of use to bumble bee conservation. Parts of London have been included in a nationwide survey (Fussell & Corbet, 1993), and Goodwin (1992, 1995) carried out a long-term (1984–1989) and detailed study of the phenology and forage preferences of bumble bees in her garden in south-west London. However, until the start of this study our knowledge of *Bombus* species occurring across London was based on data that are 20 years old (IBRA/BRC, 1980).

In the U.K. there are 391 plant and animal species with Biodiversity Action Plan status. They are assigned this status because they are nationally scarce and declining. The purpose of an action plan is to provide information on the threats the species are facing and the opportunities for maintaining and enhancing their populations. The action plans for all five species of *Bombus* express the need for further ecological research to identify their forage and habitat requirements (Anonymous, 1999). As the only B.A.P. species found in reasonable numbers in London, *B. humilis* is of particular interest to this thesis. The most comprehensive work conducted on this species has focused on populations inhabiting the expansive protected unimproved grasslands of Salisbury Plain Training Area, in Wiltshire and Castlemartin Range in South Pembrokeshire, and

has provided invaluable knowledge of its forage and habitat requirements (Carvell, 2001, 2002). The Thames Corridor population occupies a very different environment, faces some different problems and may have unique conservation management requirements. It may therefore provide added insight of use to conservation biologists that is not provided by other *B. humilis* populations. Details of the phenology of *B. humilis*, its competitive interactions with other species of *Bombus* and its large-scale population dynamics are still scarce or absent, making the development and application of a comprehensive management plan impossible.

Insect pollinators, including bumble bees, are notoriously difficult to track and, due to their size, do not lend themselves to the conventional methods used to track individuals. Consequently, their large-scale spatial foraging patterns and resource sharing at any scale are relatively poorly understood (Osborne *et al.*, 1999; Schulke & Waser, 2001; Steffan-Dewenter *et al.*, 2002). As social insects, bumble bees also pose an interesting challenge to conservation biologists when censussing populations and estimating effective population sizes (Chapman & Bourke, 2001). A bumble bee population is composed of colonies made up of many hundreds or thousands of individuals. Since most individuals within colonies are non-reproductive workers, a few queens mated to one or a few males represent the reproductive population. This situation means that the effective population size in social insects such as bumble bees may be lower than the number of individuals by many orders of magnitude (Wilson 1963; Crozier & Pamilo, 1996). This in turn means that, in principle, a population that appears numerically abundant within a habitat judging from the density of workers may be vulnerable to chance genetic and demographic effects. As a result, fundamental questions regarding

the spatial ecology of bumble bees remain unanswered, thereby hampering conservation efforts.

In this Ph.D. thesis, field observations, a field manipulation experiment and genetic analysis are successfully employed to investigate the foraging ecology and conservation biology of bumble bees in the urban habitat of London, U.K.. To fill the gaps in our knowledge described above, I conducted investigations into the distribution, foraging ecology and phenology of *Bombus* species across a range of habitats in London, a detailed investigation of the foraging ecology, phenology and competitive interactions of *B. humilis* in urban habitats, and an investigation of space use at several scales in foraging bumble bees. To my knowledge this research represents the first large-scale, systematic study of the species distribution and foraging ecology of bumble bees in any city.

Chapter 2

Species distribution and foraging ecology of bumble bees in a large urban area (London, U.K.)

SUMMARY

There are growing concerns over the loss of several bumble bee species from Britain and the decline in the abundance and range of others. In the quest for information of use to bumble bee conservation, many studies have focussed on rare species in threatened habitats and the potential of our flower-rich cities has been overlooked. The overall aim of this study was to determine the status and foraging requirements of bumble bees in the urban environment provided by the city of London, U.K.. There were three specific goals. First, to establish which species of bumble bee are found in the London area. Second, to record the abundance of the species and their castes over time. Third, to survey forage availability and identify the forage and habitat usage that different bumble bee species have within this environment. Thirty study sites were selected across London encompassing an area of approximately 700km^2. Each site was visited three times between June and August in 2000. Foraging bumble bees were identified along transects and flowering plants were surveyed using quadrats. Seven species of *Bombus* were identified during the study. Five species (*B. terrestris*, *B. lucorum*, *B. pascuorum*, *B. lapidarius* and *B. pratorum*) were found to be common but *B. hortorum* was more limited in its geographical distribution and habitat use. *B. humilis*, a Biodiversity Action Plan species, was discovered at one wasteland site in the East End of London. This is the first recorded urban location for this species. The greatest abundance of foraging workers was recorded in south London and the greatest diversity of *Bombus* species in the east. Wasteland supported the greatest abundance of workers, closely followed by gardens. Wasteland and gardens each supported six of the seven *Bombus* species. There was an equal abundance of native and introduced forage but there were significantly greater abundances of native flowers in cemeteries,

wastelands and common land. *B. pascuorum* and *B. pratorum* were the only species to forage on native species more frequently than non-native species. These species also visited perennial flowers more frequently, whereas *B. lapidarius* visited annual flowers more frequently, *B. terrestris / B. lucorum* were observed foraging on biennials more often and *B. hortorum* and *B. humilis* foraged on all three equally. Of the recorded available forage, the common wildflowers *Trifolium repens* (white clover) and *Rubus fruticosus* (blackberry) were favoured forage plants of *B. terrestris / B. lucorum*, *B. pratorum* and *B. lapidarius*. *B. pascuorum* was found to forage more frequently from the Fabaceae and Lamiaceae families. Conservation management recommendations include the sowing of wildflower seed mixtures to enhance common land and the improvement of public parks as habitat for bumble bees through the informed selection of plant varieties.

1. INTRODUCTION

On account of their role as pollinators, bumble bees are considered to be 'ecological engineers' (Jones *et al.*, 1994), altering the physical environment and affecting how resources flow through ecosystems. They also provide essential 'ecosystem services' to people (Kearns *et al.*, 1998) and are known to be particularly important pollinators of leguminous seed crops and fruit trees (Free, 1993). Although the symptoms of pollinator scarcity include reduced fruit and seed set and reduction in yields, the pollination requirements of most of the 1,800 species of wild plants in Britain are unknown (Corbet *et al.*, 1993). There are also significant gaps in our knowledge of the interactions between invertebrate pollinators and their forage (Kearns & Inouye, 1997).

The plant-pollinator mutualisms observed today are the result of approximately 100 million years of evolution (Proctor *et al.*, 1996). Despite, or perhaps because of this, examples of very tight associations between plants and their pollinators are rare (Waser *et al.*, 1996). There are a few examples of plants needing specific pollinators and even fewer examples of pollinators needing specific plants (Waser *et al.*, 1996). It is thought that only a very short colony duration enables three species of alpine bumble bee to specialize on one species or genera of flower (Goulson, 2003), but these are the exception. Some British species have been found to collect pollen from some plant species more than others (Edwards, 2001; Goulson & Darvill, 2004) but the reasons for this are currently unknown. Bumble bees are considered to be specialists, in that they obtain virtually all of their nutrition from nectar and pollen. On the whole, they are generalist pollinators and visit a vast array of flowering plant species, utilising their highly specialised mouth and body parts to extract as much nutritional gain from

flowers, with minimum energetic cost to themselves. They can be highly flexible and quick to adapt when presented with constantly changing forage options and conditions (Waser *et al.*, 1996). A complex combination of factors result in whether a bumble bee opts to forage on one flower rather than another. These include previous experience of the rewards a flower offers, the physical shape of the flowers that determines handling time, the sensory limitations that determine the detectability of the flower and possibly an innate preference (Waser & Chittka, 1998).

Of the 19 *Bombus* species recorded in Britain since 1900, two are thought to have been lost from Britain in the last sixty years (Williams, 1982, 1986), the most recent, *B. subterraneus,* being declared extinct in 1998 (WWF, 1998). Other species, particularly longer-tongued ones, are reported to have suffered a decline in numbers and range. Such is the concern over these extinctions and declines that five *Bombus* species are included in the U.K. Biodiversity Action Plan (BAP) (*B. distinguendus*, *B. humilis*, *B. ruderatus*, *B. subterraneus* and *B. sylvarum*) and three further species are included in English Nature's Species Recovery Programme (*B. monticola*, *B. muscorum*, *B. ruderarius*) (Anonymous, 1995, 1999). The main threats to bumble bees in the wild and the possible causes for their decline are outlined in the Introduction (Chapter 1 of this thesis). Natural threats include predation and attack from parasitoids. Man-made threats include the destruction of the bumble bees' natural over-wintering, nesting and foraging habitats, particularly through agricultural practices. The decline of British bumble bees has been particularly severe in the intensely arable east-central region, where agricultural intensification is blamed for the reduction of forage in the form of perennial herbaceous vegetation (Osborne *et al.*, 1991; Williams, 1982). As it is

situated in the south east of England and displays a rich and diverse flora, London could be providing, or in future provide, a refuge for species declining elsewhere. Until the start of this study our knowledge of *Bombus* species distribution within London was based on data that are 20 years old (IBRA/BRC, 1980).

Urban areas consist of a variety of components ranging from totally artificial, built environments, to semi-natural areas. The magnitude and nature of the changes in the physical, chemical and biotic environments associated with urbanisation are only just beginning to be understood. Challenges faced by urban flora and fauna include, for example, a wide range of microclimates, high levels of air, water and soil pollutants, resource patchiness and regular habitat disturbance. High proportions of exotic and naturalised species and a lower representation of native species are also features of an urban environment (McDonnell & Pickett, 1990). Despite this, native biodiversity does survive, and sometimes thrive (Owen & Owen, 1975). For example, a recent experimental study has shown that bumble bee nests placed in suburban gardens had a greater cumulative weight gain than those placed in conventional farmland and even farmland with substantial areas of set-aside (Goulson *et al.*, 2002). In another study, the city of Berlin was found to support 262 species of bees, nearly half the species total for the whole of Germany (Saure, 1996).

Greater London covers an area of almost 158,000 hectares (over 600 square miles), more than 40% of which is green open space, half of which in turn is considered valuable as wildlife habitat (London Biodiversty Partnership, 2000b). This 'green space' falls into two broad categories: space that is 'wild' and receives a minimum

amount of management and space that is managed to a high degree. Private gardens and many public spaces are managed to be florally attractive throughout the year and therefore have the potential to be hugely beneficial to foraging bumble bees. However, there is concern that, whilst they look forage-rich, these areas actually support a high proportion of modern garden cultivars. Through their intensive modification many varieties of flowering plants have lost their ability to produce nectar or have developed a physical form that no longer allows the corolla to be accessed by pollinators (Comba *et al.*, 1999a, 1999b; Corbet *et al.*, 2001). Urban areas also support large numbers of introduced species of flowering plants that have evolved in the absence of our native pollinators and may therefore not be compatible with them. The urban floral succession is almost entirely manipulated by man. The species planted are not necessarily selected for their benefits to wildlife but may be selected, for example, on the basis of their colour or cost alone in the absence of any other incentive. Should information on the qualitative 'value' of a species or variety of plant to pollinators, as well as information on the pollinators' preferences and dislikes, become available to site managers and gardeners, then urban habitats could be manipulated in favour of a range of pollinators, including bumble bees.

The overall aim of this study was therefore to determine the status and forage usage of bumble bees in the urban environment provided by the city of London, in order to provide information of use for bumble bee conservation. There were three specific goals. First, to establish which species of bumble bee are found in the London area and to determine their distribution within the area through a combination of a search for existing unpublished data and a practical survey. Second, to record the relative

abundance of the species and the succession of their castes over time (phenology) through fieldwork. Third, to survey forage availability over time and investigate the forage and habitat usage, in the field, that different bumble bee species may have.

There is a large body of work exploring the forage requirements of bumble bees. The majority of this work has been carried out either in experimental plots (Comba *et al.*, 1999a, 1999b; Corbet *et al.*, 2001), in agricultural environments (e.g. Dramstad & Fry, 1995; Svensson *et al.*, 2000), or in exceptional habitats such as Sites of Special Scientific Interest (Carvell, 2001, 2002; Williams, 1988). Although some research has been carried out in suburban areas (Goulson *et al.*, 2000), cities (Saure, 1996), and even in London (Fussell & Corbet, 1993; Goodwin, 1992, 1995), to my knowledge this is the first large-scale, systematic study of the species distribution and foraging ecology of bumble bees in any city. The London area was included in a nationwide survey which used volunteers to identify bumble bees and their forage (Fussell & Corbet, 1993), but this only identified bee species by their colour group and plants by their common name, thereby losing detail. Goodwin (1992, 1995) carried out a long-term (1984–1989) and detailed study of the phenology and forage preferences of London bumble bees, but this was at a single site (her garden) in south-west London.

2. MATERIALS AND METHODS

Between June and August 2000, I recorded flowering plant and bumble bee species along 1m × 100m transects at thirty study sites across London. Available forage was surveyed at 15m intervals along each transect using $1m^2$ quadrats and bumble bee species were surveyed continuously using the 'bee walk' method (as in: Saville *et al.*, 1997). All surveys were completed between 09:30 and 18:00, in dry weather and when temperatures were over 15°C.

Without practice bumble bees can be difficult to identify to species in the field. Therefore, prior to the start of the main study, I collected worker bumble bees, created a reference collection and identified specimens to species using the key in Prys-Jones and Corbet (1991). Paul Williams of the Department of Entomology, Natural History Museum, critically examined the collection and highlighted potential pitfalls. During the 'bee walks' I identified bumble bees while they were feeding. If there was a question over their identity then individuals were captured and identified using a hand lens, particularly when two species of similar appearance could be present. It was impossible to capture and examine every worker of the two abundant species *B. terrestris* and *B. lucorum*, whose workers closely resemble one another, so observations of these species were pooled for analysis. (By the end of this field season I was able to distinguish between workers of the two species, in the field, and collect just the *B. terrestris* workers required for the genetics work described in Chapter 5 of this thesis.) The males and queens of *B. terrestris* and *B. lucorum* are distinctive and so were identified in the field and recorded separately. Queen bumble bees were neither caught nor killed but were collected when found dead. In addition to conducting the original field work

described below, I issued a request to museums, wildlife trusts, conservation bodies and entomologists within London for any unpublished *Bombus* records.

2.1 Selection of study sites

Five widely-occurring urban habitats were selected to be surveyed for bumble bee species and their forage. These were as follows: cemetery, common land, private garden, public park or garden, and wasteland. Potential sites were identified using Ordnance Survey maps and were visited to assess suitability for the study. The chosen sites were selected for three reasons. First, they were selected to be typical, rather than exceptional, examples of their type; second, they were easily accessible; and third, they were close to other study sites of different habitat type, hence partially controlling for differences in geographic location. Clusters of the five habitats types were replicated six times across London, yielding thirty study sites (Table 1; Figure1). At each study site I selected one transect 100m long and 1m wide. A transect was selected for three reasons. First, it provided as close as possible to a continuous distribution of flora. Second, it stood a good chance of supporting forage over the study period. Third, the wooden pegs used to mark the transect were unlikely to be moved or cause damage to people or machinery. The position of each transect was marked with wooden pegs and photographed on each visit. The quadrat locations were also semi-permanently marked using wooden pegs sunk into the ground to ensure accurate replication over time. In the event of the loss of a marker the transect could be re-measured and the quadrat relocated with confidence.

2.2 Forage availability survey

To sample the availability of potential forage I placed a $1m^2$ quadrat at 15m intervals along the 100m transect. The quadrat was sub-divided with string into one hundred $10cm^2$ units, known as squares from here on. Each square was surveyed for the presence or absence of inflorescences. Where inflorescences were present in a square they were identified to species and their presence was mapped by square onto a standardised data sheet. Each square could only contain one species of flower. On the rare occasion that two species of flower were present in one square, the dominant plant was recorded. Using this method all of the plants in flower at the time, within the quadrat, were identified to species and the area they covered within the quadrat calculated. Wildflowers were identified to species using standard floras (Blamey & Grey-Wilson, 1989; Stace, 1997). Garden plants were identified as closely as possibly to species and variety (Brickell, 1989; Scott-Macnab, 1997). Wildflower plant nomenclature follows Stace (1997) and that of garden plants follows Brickell (1989). The forage availability survey was always carried out before the 'bee walk' to ensure familiarisation with the species of plant present. This enabled me to conduct the 'bee walk' as swiftly as possible and so to minimise the double counting of individuals.

2.3 Bumble bee species survey ('bee walk')

I allowed five minutes to elapse between finishing the forage availability survey and starting the 'bee walk', to enable the bees to settle back into their foraging pattern. I noted the time at the start and finish of each 'bee walk', which was taken at a regular slow pace, i.e. of 4 – 5 minutes per 100m. If the transect ran through an open area then bumble bees were recorded within 0.5m either side of the line, but if the transect was

only accessible from one side, for example in the case of a flowerbed, then bumble bees were recorded 1m deep into the relevant area. Each bee was identified to species and caste by sight or on closer inspection with a hand lens. The bee's behaviour was noted and if it was foraging I recorded the species of flower it was visiting. Environmental variables were also recorded at the start of the bee walk (Appendix A). Each site was visited and surveyed three times, in three rounds roughly four weeks apart, except for the Prince's Gardens site which was only visited two times due to lack of accessibility in the third round (Table 1).

2.4 Statistical methods

All of the data were analysed using χ^2 tests. In many transects the total number of workers of each species recorded was low, so in order to carry out the χ^2 tests correctly the data had to be pooled in order to gain expected values greater than five. Therefore no χ^2 tests were performed on individual transects. Rather, for each round, the data of the five transects for each habitat type were pooled, so that comparisons could be carried out between habitat types. Before this was done the data were tested for homogeneity and where the χ^2 test proved not significant, i.e. the observations were distributed evenly, the data were pooled and subjected to further analysis. All χ^2 tests were applied to the count data rather than percentage data. All analyses were carried out on data for worker bees only. This was done because the majority of data collected were for workers and because to a great extent the success of the colony depends on workers' foraging success. Due to the experimental design some workers were recorded during the 'bee walk' foraging on species of plants that were not recorded in the quadrats. These foraging observations were therefore excluded from the analysis of

forage visitation frequency and represented a mean of 1.73% (range, 0 - 4.8%) of observations.

3. RESULTS

3.1 Bumble bees

Historical data

The results of the request for any unpublished *Bombus* records from London yielded few records. From the information available I concluded that the six 'mainland ubiquitous' species (*B. terrestris*, *B. lucorum*, *B. hortorum*, *B. pratorum*, *B. lapidarius* and *B. pascuorum* (Williams, 1982)) were expected to be widespread and common in London. These are the only species that have been well represented in all mainland regions since 1960 (Williams, 1982). The most interesting historical data obtained during this study were supplied by David Sheppard at English Nature and extracted from the 'Recorder' database designed to manage records of rare and threatened species. Their only Greater London records are for Hampstead Heath between 1832 and 1947 (Table 2). Due to the fact that the data are extracted from a rare and threatened species database the six 'mainland ubiquitous' species are not featured. Nine species of *Bombus* were recorded, five of which are currently U.K. Biodiversity Action Plan species (*B. distinguendus*, *B. humilis*, *B. ruderatus*, *B. subterraneus* and *B. sylvarum*). The remaining four species (*B. jonellus*, *B. muscorum*, *B. ruderarius* and *B. soroeensis*) are no longer common in Britain. *B. jonellus*, *B. muscorum*, and *B. soroeensis* are considered to be widespread but locally restricted species and *B. humilis*, *B. ruderarius*, *B. ruderatus*, *B. subterraneus* and *B. sylvarum* are southern and locally restricted species (Williams, 1982). Any of these nine species might be expected in London but in low numbers and with a limited distribution, except for *B. distinguendus*, which is now extremely rare and restricted to the extreme north of Scotland (Anonymous, 1999).

Bumble bee species overall abundance

Forty-five days were spent surveying the thirty study sites. The weather ranged from overcast to sunny and the mean temperature during survey work was 19.7 °C (range 15°C –24 °C) (Appendix A). Over this period a total of 704 bumble bees was counted, the majority of which were worker bees (Table 3). There was a significant difference in the relative abundance of species in all rounds combined (χ^2 = 296.2, d.f. = 6, P < 0.001). The following seven species of bumble bee were identified in order of overall worker abundance: *B. terrestris* and *B. lucorum* combined (*B. terrestris / B. lucorum* from here on) (53%), *B. pascuorum* (22%), *B. lapidarius* (13%), *B. pratorum* (7%), *B. hortorum* (3%), *B. humilis* (<1%). *B. humilis* was the least common species, with only four individuals being recorded in the whole study. In 2001, *B. humilis* was the subject of intensive study, the results of which are reported in Chapter 3 of this thesis.

Bumble bee phenology

There was a significant difference in the relative abundance of species in each round (Round 1: χ^2 = 161.2, d.f. = 6, P < 0.001; Round 2: χ^2 = 138.3, d.f. = 6, P < 0.001; Round 3: χ^2 = 61.4, d.f. = 6, P < 0.001). In the first two rounds, *B. terrestris / B. lucorum* workers were the most frequently observed, followed by *B. pascuorum*, but in the third round *B. pascuorum* workers were the most frequently observed, followed by *B. terrestris / B. lucorum*. The abundance of each caste varied with each round (Figure 2). In all species the number of workers was greatest in the first round (June to mid-July) and declined with subsequent rounds (χ^2 = 66.7, d.f. = 12, P < 0.001). The only queen observed in the first round was a *B. pratorum* queen and by the second round *B.*

pratorum workers were absent and only the males of this species were seen. *B. terrestris*, *B. lucorum*, *B. lapidarius* and *B. hortorum* males appeared in the second round, *B. pascuorum* males were only observed in the third round and males of *B. humilis* were not observed (Figure 2).

Bumble bee worker abundance by species and region

Species of bumble bee differed significantly in the relative abundance of their workers across the different geographical regions of London (χ^2 = 93.63, d.f. = 30, P < 0.001). This difference was also observed in Rounds 1 and 2 (Round 1: χ^2 = 90.46, d.f. = 30, P < 0.001; Round 2: χ^2 = 37.56, d.f. = 20, P < 0.01) (Figure 3). The greatest number of workers was recorded in the south (23%) followed by the north (20.5%) and the least in the west (9%). *B. terrestris / B. lucorum*, *B. pascuorum*, *B. lapidarius* and *B. pratorum* were recorded across the city, *B. hortorum* was not recorded at sites in the west and south west and *B. humilis* was only recorded in the east (Figure 3).

Bumble bee worker abundance by species and habitat

No habitat was found to contain all seven species (Figure 4). Workers of different species were observed more frequently in different habitats in all rounds combined (χ^2 = 99.2, d.f. = 24 P < 0.001) and in Rounds 1 and 3 but not in Round 2 (Round 1: χ^2 = 69.9, d.f. = 24, P < 0.001; Round 2: χ^2 = 30.5, d.f. = 16, P > 0.05; Round 3: χ^2 = 54.3, d.f. = 20, P < 0.001). All habitats supported six species, except commons, which only supported four species. *B. terrestris / B. lucorum*, *B. pascuorum* and *B. lapidarius* were recorded in all habitats. *B. hortorum* was not found on commons and wastelands, *B. pratorum* was not found on commons and *B. humilis* was only observed in the

wasteland habitat. *B. terrestris / B. lucorum*, *B. pratorum* and *B. hortorum* were observed more frequently in gardens (*B. terrestris / B. lucorum*: $\chi^2 = 149.5$, d.f. = 4, $P < 0.001$; *B. pratorum*: $\chi^2 = 30.5$, d.f. = 4, $P < 0.001$; *B. hortorum*: $\chi^2 = 18.5$, d.f. = 4, $P < 0.001$) whereas *B. pascuorum* and *B. lapidarius* were observed more frequently on wasteland (*B. pascuorum*: $\chi^2 = 37.4$, d.f. = 4, $P < 0.001$; *B. lapidarius*: $\chi^2 = 72.0$, d.f. = 4, $P < 0.001$). *B. humilis* was only found at wasteland sites.

3.2 Plants

Flowering plant abundance

One hundred and ninety four species of plant from 43 families were identified during the flower survey. A further 18 species and an additional two families were identified as forage during the 'bee walks'. In total, 110 of these species are native to the U.K. and 103 have been introduced. Thirty-two of the recorded species are annual, 16 biennial and 164 perennial. In the first round significantly more of the squares sampled contained forage than in the following two rounds ($\chi^2 = 765.7$, d.f. = 2 $P < 0.001$) and more than double the amount of available forage in the third round. This pattern of decreasing floral abundance over time was reflected in all of the habitats except for the garden habitat, where there was a marginal increase in the second round (cemetery: $\chi^2 = 111.2$, d.f. = 2, $P < 0.001$; common: $\chi^2 = 88.6$, d.f. = 2, $P < 0.001$; park: $\chi^2 = 292.8$, d.f. = 2, $P < 0.001$; wasteland: $\chi^2 = 82.2$, d.f. = 2, $P < 0.001$ garden: $\chi^2 = 1.0$, d.f. = 2, $P > 0.05$) (Figure 5). There was also a significant difference in the abundance of forage between the different habitats (Figure 5). Across all three rounds combined the garden habitat offered more forage than the other habitats ($\chi^2 = 2845.4$, d.f. = 4, $P < 0.001$). There was a significant difference in the abundance of flowers available across

different geographical areas of London (χ^2 = 526.7, d.f. = 5, P < 0.001). The greatest availability of potential forage was found in the east of London, where 55% of squares sampled contained potential forage. The west of London supported the least potential forage, with 23% of squares sampled containing potential forage.

There was a significant difference in the abundance of native and non-native flowers across the habitats (χ^2 = 3201.0, d.f. = 4, P < 0.001). Cemeteries, commons and wasteland were dominated by native plant species (cemeteries: χ^2 = 756.5, d.f. = 1, P < 0.001; commons: χ^2 = 903.1, d.f. = 1, P < 0.001; wasteland: χ^2 = 47.6, d.f. = 1, P < 0.001), whereas gardens and parks were found to have a greater abundance of introduced species (gardens: χ^2 = 956.1, d.f. = 1, P < 0.001; parks: χ^2 = 582.6, d.f. = 1, P < 0.001) (Figure 7). Overall there was a significantly greater abundance of perennial plants than of annuals, biennials and shrubs (χ^2 = 9476.4, d.f. = 3, P < 0.001). Perennial flowers dominated all habitats except for gardens, which had a marginally greater number of flowering shrubs.

Flowering plant diversity

Just fewer than two hundred species of plants in flower were recorded across all of the study sites. There was no significant difference in floral diversity between the rounds. In all three rounds the number of species identified was greatest in the wasteland habitat and least in the common habitat (Round 1: χ^2 = 17.19, d.f. = 4, P < 0.01; Round 2: χ^2 = 24.12, d.f. = 4, P < 0.001; Round 3: χ^2 = 20.63, 4 d.f. = 4, P < 0.001) (Figure 8). There was a significant difference in the plant species diversity of the study sites (χ^2 = 105.0, d.f. = 29, P < 0.001). Two sites stood out as the most floristically rich (Table 4a).

These are Sand's End in the Borough of Fulham and Ferndale Street in the Borough of Newham, both of which are wasteland sites. In total, 33 species of flowering plant were recorded at both Sand's End and Ferndale Street, whereas only seven species each were recorded at St. Luke's Church, Chelsea and Hampstead Heath (Table 4b). There was no significant difference in the diversity of flowers available across different geographical areas of London ($\chi^2 = 3.4$, d.f. = 5, $P > 0.05$).

Forage visitation frequency of bumble bee workers

Bumble bee workers were observed foraging on 61% of the flower species recorded. Each species of bumble bee worker foraged more frequently on a different species of flower, but *Rubus fruticosus* (blackberry), *Lamium album* (white dead nettle) and *Trifolium repens* (white clover) had the broadest appeal and were the most frequently featured in the top ten flowers for each species of bumble bee (Table 5). Worker bees from all species were observed foraging on some plant species more often than others and did not just visit them in proportion to their abundance (*B. terrestris / B. lucorum*: $\chi^2 = 1520.4$, d.f. = 193, $P < 0.001$; *B. pascuorum*: $\chi^2 = 1015.8$, d.f. = 193, $P < 0.001$; *B. pratorum*: $\chi^2 = 956.1$, d.f. = 193, $P < 0.001$; *B. lapidarius*: $\chi^2 = 673.8$, d.f. = 193, $P < 0.001$; *B. hortorum:* $\chi^2 = 646.1$, d.f. = 193, $P < 0.001$; *B. humilis*: $\chi^2 = 242.3$, d.f. = 193, $P < 0.001$). Some plants were not very common but attracted a disproportionate number of foraging worker bees. Conversely, workers ignored several species of flowering plant even though they were abundant. For example, *B. terrestris / B. lucorum* were attracted to the uncommon *Geranium dissectum* and *Philadelphus* var. but ignored *Penstemon* var. Similarly, *B. hortorum* was frequently observed foraging on *Gentiana asclepiadea* and *Cerinthe major* 'purpurascens' but avoided *R. fruticosus*

(Table 6). Of the top ten most abundant flowers, four species were never observed hosting foraging workers of any species. These were: *Penstemon* var., *Potentilla fruticosa*, *Dahlia* var. and *Hydrangea macrophylla*. *B. terrestris / B. lucorum* were the only species observed foraging on *Tagetes* 'Tangerine dream', despite it being common, and all *Bombus* species except *B. pascuorum* avoided *Pelargonium* var.

The only bumble bee species observed to forage more frequently on native forage species were *B. pascuorum* and *B. pratorum* (*B. pascuorum*: $\chi^2 = 9.9$, d.f. = 1, $P < 0.01$; *B. pratorum*: $\chi^2 = 8.2$, d.f. = 1, $P < 0.01$). Worker bumble bees did not necessarily visit annual, biennial and perennial plants or shrubs in proportion to their abundance. *B. lapidarius* visited annual plants more frequently ($\chi^2 = 16.4$, d.f. = 3, $P < 0.001$), *B. terrestris / B. lucorum* visited biennials more frequently ($\chi^2 = 48.3$, d.f. = 3, $P < 0.001$) and *B. pascuorum* and *B. pratorum* visited perennial flowers more frequently than the other classifications (*B. pascuorum*: $\chi^2 = 22.5$, d.f. = 3, $P < 0.001$; *B. pratorum*: $\chi^2 = 8.4$, d.f. = 3, $P < 0.1$). *B. hortorum* and *B. humilis* were observed visiting annual, biennial and perennial plants in equal proportions.

4. DISCUSSION

In a survey of five urban habitat types spread over thirty sites in London, I identified six 'mainland ubiquitous' bumble bee species and one Biodiversity Action Plan species, *B. humilis*. Five 'mainland ubiquitous' species were found to be common and widespread, but one of them, *B. hortorum*, was much less frequently encountered and had a limited distribution across the city and habitats. The Biodiversity Action Plan species, *B. humilis*, was identified from one wasteland site in the East End. The greatest abundance of foraging workers was recorded in south London and the greatest diversity of bees in the east. Of all of the habitats wasteland supported the greatest abundance of workers, closely followed by gardens, and wasteland and gardens each supported six of the seven *Bombus* species. Overall there was an equal abundance in the availability of native and introduced flowers but there were significantly more native flowers in cemeteries, wastelands and common land. *B. pascuorum* and *B. pratorum* were the only species of bumble bee to visit native species more frequently and they also visited perennial flowers more often than flowers of annuals, biennials or shrubs. *B. lapidarius* visited annual flowers more frequently, *B. terrestris / B. lucorum* visited biennials more frequently and *B. hortorum* and *B. humilis* showed visited annuals, biennials, perennials and shrubs equally. Of the selection of forage available, the common wildflowers *Trifolium repens* (white clover) and *Rubus fruticosus* (blackberry) were the most frequently utilised forage plants of *B. terrestris / B. lucorum*, *B. pratorum* and *B. lapidarius*. *B. pascuorum* was found to forage from the Fabaceae and Lamiaceae families more often than other families of plants.

At this point limitations in the survey method should be noted. We can rarely carry out a complete census of organisms in area, due to restrictions in time, manpower and funding. To over come this, the abundance of an organism, for example, can be estimated by sampling. This should be conducted without bias, which would lead to an over or underestimation the mean. One of the most common sources of bias is the selection of sample plots that are non-random, with respect to the abundance of the organism in question. A random sample is one where every potential sample plot within the area has an exactly equal chance of being sampled. The selection of random sample plots is generally carried out with the aid of a random number generator. This was not the case in this project, as there were selection criteria, outlined above (2.1 selection of study sites). Due to the nature of the environment being surveyed, the position of a transect measuring 100m long and 1m wide was often dictated by the site, for example a flower bed in a garden. The establishment of the transects and the first survey round of took place in June. Further rounds of surveys took place in July and August. It is difficult to assess the potential forage availability of a transect and it is certain that forage availability will not stay constant over a period of time and a flower bed that provides forage in June may not do so in August. Therefore one of the selection criteria was that the area within a transect stood a good chance of supporting forage over the study period. The method of selection of transects may have been the reason for there being significantly more forage being available in the first round, than in the subsequent two rounds. Additionally, by employing fixed transects, I immediately limited the selection of species of flowering plant available for bumble bees to be observed foraging on. Furthermore, by employing an observational technique I can only learn what bumble bees are utilising at one site at one point in time. Therefore, I

have only observed the forage choices bumble bees make on a given selection of flowers, in a given habitat at a given time. Clearly I have not managed to record the entire selection of flowers available to bumble bees, neither have I recorded every species of flowering plant that bumble bees forage on. As with the majority of such studies, this survey is restricted in temporal and spatial dimensions and will therefore underestimate the forage species utilised by bumble bees. The complex interspecific and environmental interactions that lead to a bumble bee making a choice to forage on one flower or the next are not assessed. When a bumble bee is observed foraging on a flower I cannot infer preference because it may not necessarily be the species they would select given the choice of every flowering plant in the world in optimum condition. However, in reality they do not have such a choice and are very much constrained by forage availability. An urban area, such as London, does provide pollinators with a wider choice of forage than many other habitats in the U.K. Therefore, the frequency with which different species of bumble bee visit different species of flower provides valuable information to individuals and organisations aiming to contribute to bumble bee conservation through forage habitat enrichment.

Bumble bee species overall abundance

During the current study only the three species, *B. terrestris / B. lucorum* and *B. pascuorum*, were identified from the 'bee walks' on Hampstead Heath. *B. lapidarius* and *B. pratorum* were later identified during a more extensive search for the rarer species. Nevertheless, despite still supporting an impressive flora in places, Hampstead Heath has clearly suffered a significant depletion in its *Bombus* fauna since the

historical records in Table 2 were made, demonstrating that the nationwide decline in bumble bees is not just restricted to agricultural areas.

Although six out of the seven bumble bee species identified in this study are considered to be the most common and widespread in the U.K. (Prys-Jones & Corbet, 1991; Williams, 1982), it is very encouraging that they were all found in London. This means that they are finding not only sufficient forage but also sufficient nest sites. Five species were ubiquitous in London (*B. terrestris*, *B. lucorum*, *B. pratorum*, *B. lapidarius* and *B. pascuorum*) but *B. hortorum* was not encountered as frequently. This is not especially surprising as it has smaller colonies (Sladen, 1989) and completes its colony cycle more rapidly than the other species (Prys-Jones & Corbet, 1991). It is therefore predisposed to under-sampling in a survey of this nature, as noted by Benton (2000). However, care must be taken not always to attribute the apparent scarcity of *B. hortorum* to its small colony size and rapid colony cycle, as these two features may in fact make its population more susceptible to perturbations. *B. hortorum* is known to be declining in mainland Europe (Kwak *et al.*, 1996).

Bombus pratorum is known to be the earliest of the British bumble bee species to emerge from hibernation (Benton, 2000; Prys-Jones & Corbet, 1991; Sladen, 1989). By the time I had started the survey the colony cycle of this species was coming to an end and I was unable to detect any peak in worker numbers. This species was therefore under-represented in this survey due to the timing of the survey rather than due to the species being uncommon.

The discovery of *Bombus humilis* in a truly urban environment is the most exciting finding of this study. This species has suffered a particularly severe decline in recent decades and as a result has been given B.A.P. status (Anonymous, 1995, 1999). Currently it exists as a number of small isolated populations along the coast of southern Britain and Wales and at a few inland sites associated with large areas of unimproved chalk grassland . Little can be concluded from the four sightings of this species in 2000 but it is reassuring that the sampling technique was successful in detecting a rare species with specific habitat requirements. These four sightings provided a starting point for the search for *B. humilis* in 2001, when it was identified from a further eleven sites (Chapter 3 of this thesis). Although the discovery of *B. humilis* in the east of London is exciting it is not entirely surprising. Healthy populations of *B. humilis* are known from Kent and Essex and at the mouth of the River Thames in Rainham Marshes and Havering . Prior to the start of this study, Rainham Marshes marked the most western, and therefore most urban, extent of its range in the Thames Corridor. It is likely that the east London population is part of a south-east coast metapopulation but genetic studies would be needed to clarify this (e.g. Chapman *et al.*, 2003). The existence of *B. humilis* within the study area provides encouragement that London has potential to provide a refuge for species declining elsewhere. *B. humilis* ecology is discussed in detail in Chapter 3 of this thesis.

Bumble bee phenology

The phenologies described in this study are not directly comparable to the relevant ones in the literature (Goodwin, 1995; Prys-Jones & Corbet, 1991). This is because of the differences in the time scale over which the data were collected and in the way that the

data were subsequently collated. In the case of Goodwin , counts were made twice daily, and, in the case of Prys-Jones (1991), approximately weekly. This study shows worker numbers of all species peaking in the first round (03/06/01–14/07/01), whereas the studies of Goodwin and Prys-Jones detected more subtle temporal variations, showing, for example, *B. terrestris / B. lucorum* and *B. pascuorum* worker numbers peaking approximately two weeks after those described in this study. This difference may be a true one caused by natural annual variation or it may be due to analytical constraints. A more intensive comparative study, recording bee numbers on a weekly basis and over many seasons would be needed to detect any differences between urban bee phenology and that of more rural populations of the same species. Further analysis and discussion of *Bombus* phenology from data collected in 2001 appear in Chapter 3 of this thesis.

Bumble bee worker abundance by species and region

Five of the six common species were found in all of the regions of London. Given that *B. hortorum* is generally the least abundant of the six common species and given that the west of London was found to support the least bees, it is not surprising that *B. hortorum* was not recorded in the west. It is surprising, however, that it was undetected from the south-western region which is a relatively affluent area characterised by large gardens. *B. hortorum* was sighted in south-west London during the study period (but not during the formal 'bee walks') and has been recently recorded there by other observers (Goodwin, 1992, 1995; Morris, 1997), so it is not locally absent; but this finding does suggest that *B. hortorum* occurs at lower densities in this area than in the rest of London, where the sampling technique detected it.

Bumble bee worker abundance by species and habitat

Of all the habitats, commons were the least valuable to foraging workers. They supported the least abundant and diverse forage and as a result contained the lowest number of foragers from only four species of bumble bee. This is not to say that this habitat has no value to bumble bees as it may well provide valuable nest sites. Every other habitat was found to support six out of the seven species of bumble bee. The absence of *B. hortorum* from the wasteland habitat is surprising. This is our longest-tongued species and therefore requires flowers with very deep corollas, some of which, for example *Trifolium pratense*, were recorded at wasteland sites. It was observed more frequently in gardens than any other habitat, thereby living up to its reputation and name (Benton, 2000; Prys-Jones & Corbet, 1991; Sladen, 1989). *B. humilis* was only observed in the wasteland habitat. Although this species traditionally thrives on large expanses of unimproved grassland , the population identified from the Thames Corridor is known to frequent wasteland or brownfield sites .

It is interesting that the species identified in this study can be split into two broad but not mutually exclusive groups; those that were observed most frequently in gardens and those that were observed most frequently at wasteland sites. *B. terrestris / B. lucorum*, *B. pratorum* and *B. hortorum* were found more often in gardens. *B. pascuorum*, *B. lapidarius* and *B. humilis* were observed more often on wasteland.

Flowering plant abundance

When study sites were being sought at the beginning of 2000, the west of London offered so little 'green space' that it proved to be the most difficult area in which to identify suitable representatives of all five habitats. For example, wasteland sites were rare and cemeteries were of the highly manicured variety. Therefore it is not surprising that this area offered the least available forage. Conversely the east of London has more open space and relatively abundant (but ever decreasing) wasteland sites. Cemeteries were wilder but not overgrown and the municipal parks of this area were particularly rich in flower beds. As a result east London was found to provide the greatest amount of potential forage. The fact that gardens supported the most forage is to be expected as gardens are actively managed to exhibit flowers, but it does highlight the fact that with the right guidance gardens are the habitat that can be most easily manipulated to favour pollinators. Given that this study surveyed an urban area, it was surprising that almost 52% of all flowering plants recorded were species native to Britain. The most intensively managed habitats, gardens and parks, were unsurprisingly dominated by exotic species.

Flowering plant diversity

Although gardens were found to support the greatest abundance of forage, wastelands were found to offer the greatest diversity. These are areas that were once developed but have become derelict and currently support semi-natural vegetation that has established itself subsequent to previous development. Therefore to a great extent the land has been left to a natural colonisation from native wildflowers and garden escapees, which can result in a diverse, flower-rich community. Of all of the habitats surveyed, wasteland is

clearly the most at risk as it is only a matter of time before these small plots of valuable land are developed. There is a growing body of work championing the importance of wasteland habitat to a variety of wildlife as well as to the flora itself. In a recent study, Chipchase (1999) found 7% of the U.K. flora to occur in derelict sites of London alone.

Use of forage by bumble bee workers

That different species of bumble bees utilise different species of forage, thereby reducing interspecific competition, is well documented (e.g. Barrow & Pickard, 1984; Ranta & Lundberg, 1980; Teräs, 1976). This can be mainly attributed to the fact that bumble bee species have evolved different tongue lengths, enabling them to specialise in different species of flowers with corresponding corolla depths. Fussell and Corbet, (1993) conducted a nation-wide study to survey bumble bee forage plants in the U.K. This encompassed the whole of the United Kingdom, including London, and recorded an enormous 33,000 flower visits. The current study concurs with that of Fussell (1993) in many ways. For example the common wildflowers *Trifolium repens* (white clover) and *Rubus fruticosus* (blackberry) were most frequently visited by the shorter-tongued species *B. terrestris / B. lucorum*, *B. pratorum* and *B. lapidarius* (Table 5). In the present study, three out of the top four most frequently visited forage plants of *B. lapidarius* are yellow (Table 6) and Fussell (1993) also observed that this species has a propensity for foraging on clustered, yellow flowers. It must be noted that when presented with a choice of flower colours, under experimental conditions, *B. lapidarius* did not show a preference for yellow flowers (Chittka *et al.*, 2001). As with the six other species of bumble bees tested, their preference was for flowers of violet-blue colour. This suggests that rather than preferring yellow flowers, *B. lapidarius* learn that

these types of flowers are their best forage option, given their competitive environment. Fussell (1993) further noted that knapweeds were an important forage for *B. lapidarius* and in the present study two species of knapweeds were identified in the top ten flowers most visited by *B. lapidarius* (Table 5). In the present study, eight of the ten plants most visited by the longer-tongued *B. pascuorum* are from the deeper-corollaed Fabaceae and Lamiaceae families, but *B. pascuorum* was also observed foraging on very open flowers such as thistles and blackberry. The two plants that received the most visits in the study of Fussell (1993) were *Lamium album* (white dead-nettle) and *Rubus fruticosus* (blackberry). *Digitalis purpurea* (foxglove) and *Lonicera periclymenum* (honeysuckle) were recorded as being frequently visited by *B. hortorum* (Tables 5, 6) in both studies. The only species identified as *B. hortorum* forage in this study that was not recorded in that of Fussell (1993) is *Ceratostigma willmotianum.*

The present study employs a subsampling technique to identify potential forage species and to estimate the relative coverage of forage available. The method used to estimate the coverage of forage available is a crude one, but appropriate for such a large-scale study. Quantifying resources available more accurately, at a finer scale, could improve this method. Given that it is each inflorescence that provides nectar and the density of inflorescences per plant varies between species, knowing the number of individual inflorescences per area would help quantify the nectar available to a forager. In their study to examine the movements of bumble bees within and between plant species in a meadow Chittka *et al.* (Chittka *et al.*, 1997) counted the number of inflorescences available in a 8m × 20m area. They also recorded the duration each individual bee spent at an inflorescence. In the present study recording the visitation frequency and duration

to each inflorescence would give a more detailed picture of how different bumble bee species exploit different plant species.

To enable us to interpret the foraging behaviour of pollinators at an even finer scale we need to be able to quantify the currencies of foraging. Nectar is composed largely of sugar and water, and it is the sugar that provides the energy a bumble bee needs to provision the nest. The quality and quantity of the nectar resources available from one flower varies greatly over time, with the weather, the microclimate and with the age of the flower. The rate at which it is reabsorbed by the flower or removed by foragers clearly has an impact too (Corbet, 2003). In order to assess the value of a flower as a resource one needs to know how much energy it can provide (Heinrich, 1979). Biologists can employ a variety of methods to sample nectar and measure its volume, measure solute concentration in a nectar sample, and quantify standing crop and secretion rate of nectar (Corbet, 2003; Kearns & Inouye, 1993). In a recent study the combination of the quantification of nectar rewards and the visitation frequency was successfully employed to explain the behaviour of bumble bees and assess the ecological consequences of their behaviour. *Imaptiens glandulifera* (Himalayan Balsam) was found to offer a greater rate of sugar production than any plant native to central Europe. Responding to this lure, bumble bees were found to favour *I. glandulifera* over native plants, resulting in a reduction in fitness of native flora (Chittka & Schürkens, 2001).

In the literature the quantification of pollen as a currency has not been explored as thoroughly as nectar. Pollen is collected by bumble bees, not as an energy source, but

as the sole source of protein for the developing larvae in the nest. Therefore, unlike nectar, the benefits and costs of foraging cannot be directly compared and the economics of nectar collection cannot be applied to pollen. Bumble bees have been shown to collect pollen from a subset of the plant species available to them. To understand this process Rasheed & Harder (1997) measured the pollen standing crop, grain volume and protein content of the plants that were visited. They concluded that pollen-foraging bumble bees do not assess plant species based solely on intrafloral characteristics. Rather, they can assess protein availability at the site as a whole, calculate foraging costs and make economic decisions accordingly (Rasheed & Harder, 1997). The quantification of nectar and pollen resources outlined above is clearly a time consuming process. At the start of the present study the aim was to collect data on whether each bee observed was collecting nectar or pollen. I soon realised that in the short time that an observer has with a bumble bee it is possible to observe if a worker is extracting nectar from an inflorescence, but it is practically impossible to ascertain whether it is collecting pollen from that inflorescence. In order to do this satisfactorily one must employ pollen analysis of either pollen loads collected from the baskets or from the nest itself.

Conservation and management recommendations

The fact that no single habitat was found to support all seven species of bumble bee highlights the importance of the diversity of habitats that is provided by urban areas. It also shows that one habitat type cannot be put 'aside' to achieve bumble bee conservation. Wastelands were found to offer not only the greatest diversity of flowers but also species that were not found in any of the other habitats. This underlines the need for the protection of this unique habitat, not only for bumble bees, but also for other urban wildlife. Currently there are no wasteland sites with statutory site protection but the recent production of London's Habitat Action Plan for Wasteland will help to achieve this (London Biodiversity Partnership, 2001b).

The two habitats that have the greatest potential to be improved for bumble bees are public parks and common land. As areas of public parks are planted anyway, it would not necessarily take any extra effort or cost to ensure that their resource potential is not wasted with beds of varieties of flowers such as *Pelargonium* and *Tagetes* that are of little use to bumble bees. Although some important work has been conducted to investigate the value of some varieties of horticultural cultivars to bumble bees (Comba *et al.*, 1999a, 1999b), this needs to be developed further and disseminated appropriately in order to give garden managers the information that they require. The poor floral diversity and low floral abundance of common land could be improved as bumble bee forage habitat through the creation of wildflower margins. There were not enough areas of wildflowers to be included as a habitat type in this study, so there is no empirical evidence to show that they attract more bumble bees than any of the other habitats. However, the abundance and diversity of bumble bees at the wildflowers margins of

Regent's Park were notable from casual observations and the Thames Barrier Park wildflowers were shown to attract more *B. terrestris* workers than the other sites sampled (Chapter 5 this thesis). Work carried out to investigate the impact of perennial wildflower margins has shown that they benefit not only bumble bees, but also other insect pollinators such as butterflies, solitary Hymenoptera and some Diptera (Carreck *et al.*, 1999; Feber *et al.*, 1996; Kells *et al.*, 2001; Lagerlof *et al.*, 1992). An aim of current work on the restoration of bumble bee habitat on arable farmland is to develop and sow pollinator 'friendly' wildflower seed mixtures (Edwards, 2002b; MAFF, 1998, 1999). There is clearly potential for this knowledge to be applied to urban environments and for seed mixes to be adapted to suit the requirements of the managers of such environments.

Although local authorities have the potential to turn every public area into a bee haven, bumble bee conservation can be practised on an individual level and everyone with so much as a window-box planted with lavender can play their part. Private gardens make up the single greatest land type in Greater London, occupying approximately 20% (31,600ha) of the total area (London Biodiversity Partnership, 2000a). When considering urban areas as a refuge for bumble bees, this is a very important fact. No single flower species could be identified as providing the best bumble bee forage, as each bee species exhibited variations in the frequency with which they visited different species of plant. However, if the reason for *B. hortorum* being so uncommon in this study is a lack of food, then this species would benefit from the planting of its most commonly utilised flowers with deep corollas. Such species are, for example, foxgloves and honeysuckles.

Table 1. Thirty study sites visited showing their locations, their habitat types and the dates they were surveyed.

Study site (postcode)	Grid reference	Habitat type	Date visited Round 1	Date visited Round 2	Date visited Round 3
St. James Lane (N10)	TQ288895	Garden	10/06/00	18/07/00	23/08/00
Prince's Gardens (W3)	TQ192817	Garden	06/06/00	13/07/00	n/a
Queensmill Road (SW6)	TQ236771	Garden	03/06/00	16/07/00	13/08/00
Berrylands (KT5)	TQ191675	Garden	15/06/00	20/07/00	18/08/00
Pymers Mead (SE21)	TQ324733	Garden	29/06/00	22/07/00	21/08/00
Brooks Avenue (E6)	TQ427823	Garden	27/06/00	22/07/00	22/08/00
Regent's Park (NW1)	TQ277833	Park	07/06/00	18/07/00	18/08/00
Walpole Park (W13)	TQ172802	Park	06/06/00	27/07/00	19/08/00
St. Luke's Church (SW3)	TQ272781	Park	03/06/00	16/07/00	01/08/00
St. Andrew's Square (KT6)	TQ177672	Park	15/06/00	20/07/00	18/08/00
Sexby Gardens (SE22)	TQ348750	Park	28/06/00	21/07/00	15/08/00
West Ham Park (E7)	TQ402840	Park	10/07/00	26/07/00	22/08/00
Highgate (N6)	TQ286870	Cemetery	05/06/00	17/07/00	04/08/00
Hanwell (W7)	TQ157802	Cemetery	14/07/00	27/07/00	19/08/00
Brompton Cemetery (SW10)	TQ258776	Cemetery	03/06/00	16/07/00	01/08/00
Morden (KT3)	TQ229678	Cemetery	26/06/00	25/07/00	10/08/00
Nunhead (SE15)	TQ354755	Cemetery	27/06/00	24/07/00	15/08/00
Woodgrange Park (E12)	TQ418852	Cemetery	03/07/00	26/07/00	07/08/00
Hampstead Heath (NW3)	TQ264864	Common	12/06/00	17/07/00	04/08/00
Ealing Common (W5)	TQ185804	Common	14/07/00	27/07/00	19/08/00
Barnes Common (SW15)	TQ226764	Common	15/06/00	20/07/00	09/08/00
Cannon Hill Common (SW20)	TQ238683	Common	26/06/00	25/07/00	10/08/00
Burgess Park (SE5)	TQ332777	Common	29/06/00	22/07/00	15/08/00
Wanstead Flats (E11)	TQ405865	Common	07/07/00	26/07/00	22/08/00
Tollington Road (N7)	TQ311861	Wasteland	03/07/00	24/07/00	04/08/00
Jenner Avenue (W3)	TQ207815	Wasteland	12/07/00	27/07/00	09/08/00
Sand's End (SW6)	TQ260767	Wasteland	06/06/00	12/07/00	13/08/00
Tolworth (KT5)	TQ198655	Wasteland	26/06/00	25/07/00	09/08/00
Ossory Road (SE1)	TQ340779	Wasteland	28/06/00	21/07/00	21/08/00
Ferndale Street (E6)	TQ435809	Wasteland	06/07/00	26/07/00	07/08/00

Table 2. A summary of rare *Bombus* species recorded on Hampstead Heath between 1832 and 1947, extracted from the 'Recorder' database records and provided by David Sheppard at English Nature in 2000. BAP denotes a 'Biodiversity Action Plan Species'. SRP denotes an 'English Nature Species Recovery Programme Species'.

Species name	Current status	Date recorded	Source of record
B. subterraneus	BAP	10/06/1917	Andrewes, Sir C.H.
		27/08/1918	Andrewes, Sir C.H.
		28/07/1917	Bradley, R.C.
		1836	Saunders. E
B. distinguendus	BAP	1836	Spooner, G.M.
		1832 - 1947	Guichard, K.M.
B. ruderatus	BAP	1836	Spooner, G.M.
		1832 - 1947	Guichard, K.M.
		02/05/1920	Andrewes, Sir C.H.
		11/07/1920	Andrewes, Sir C.H.
		01/07/1916	Andrewes, Sir C.H.
B. humilis	BAP	1832 - 1947	Guichard, K.M.
B. sylvarum	BAP	1836	Spooner, G.M.
B. muscorum	SRP	1836	Spooner, G.M.
B. soroeensis		1836	Spooner, G.M.
B. jonellus		1836	Spooner, G.M.
B. ruderarius	SRP	1836	Spooner, G.M.
		1832 - 1947	Guichard, K.M.

Table 3. Summary of bumble bees, by caste and species, identified foraging in each round.

Caste	Species	Round 1 (03/06/00 – 14/07/00)	Round 2 (12/07/00 – 27/07/00)	Round 3 (01/08/00 – 23/08/00)	Total
Worker	*B. terrestris / B. lucorum*	184	107	19	310
	B. pascuorum	57	45	28	130
	B. lapidarius	35	23	16	74
	B. pratorum	42	0	0	42
	B. hortorum	10	7	3	20
	B. humilis	3	0	1	4
	Total workers	**331**	**182**	**67**	**580**
Male	*B. terrestris*	1	6	17	24
	B. lucorum	3	6	10	19
	B. pascuorum	0	0	24	24
	B. lapidarius	4	13	20	37
	B. pratorum	1	10	0	11
	B. hortorum	0	3	0	3
	B. humilis	0	0	0	0
	Total males	**9**	**38**	**71**	**118**
Queen	*B. terrestris*	1	1	2	4
	B. lucorum	0	0	0	0
	B. pascuorum	0	0	0	0
	B. lapidarius	0	0	0	0
	B. pratorum	1	0	0	1
	B. hortorum	0	1	0	1
	B. humilis	0	0	0	0
	Total queens	**2**	**2**	**2**	**6**
	Total bees	**342**	**222**	**140**	**704**

Table 4a. Plant species diversity at each transect, shown in descending order according to floral diversity in each round.

Habitat: Ce = cemetery, Co = common land, G = garden, P = park, W = wasteland. Region: N = North, E = East, S = South, SW = South West, W = West, C = Central.

Round 1 Location Name	Habitat	Region	No. of plants	**Round 2** Location Name	Habitat	Region	No. of plants	**Round 3** Location Name	Habitat	Region	No. of plants
Ferndale Street	W	E	16	Sand's End	W	C	16	Sand's End	W	C	16
Ossary Road	W	S	13	Tolworth	W	SW	14	Ferndale Street	W	E	14
Jenner Avenue	W	W	12	St. James' Lane	G	N	12	Burgess Park	P	S	10
St. James' Lane	G	N	12	Burgess Park	P	S	11	Ossary Road	W	S	10
Tolworth	W	SW	11	Ferndale Street	W	E	11	St. James' Lane	G	N	10
Tollington Road	W	N	10	Jenner Avenue	W	W	11	Tolworth	W	SW	10
Burgess Park	P	S	9	Ossary Road	W	S	10	Tollington Road	W	N	9
Highgate Cemetery	Ce	N	9	Tollington Road	W	N	10	Jenner Avenue	W	W	8
Berrylands	G	SW	8	Woodgrange Park Cemetery	Ce	E	10	Woodgrange Park Cemetery	Ce	E	8
Morden Cemetery	Ce	SW	8	Sexby Gardens	P	S	9	Highgate Cemetery	Ce	N	7
Regent's Park	P	N	8	Hanwell Cemetery	Ce	W	8	West Ham Park	P	E	7
Ealing Common	Co	W	7	Morden Cemetery	Ce	SW	8	Barnes Common	Co	C	6
Sand's End	W	C	7	Brompton Cemetery	Ce	C	7	Hanwell Cemetery	Ce	W	6
Walpole Park	P	W	7	Pymers Mead	G	S	7	Morden Cemetery	Ce	SW	6
Woodgrange Park Cemetery	Ce	E	7	West Ham Park	P	E	7	Pymers Mead	G	S	6
Nunhead Cemetery	Ce	S	6	Cannon Hill Common	Co	SW	6	Queensmill Road	G	C	6
Sexby Gardens	P	S	6	Ealing Common	Co	W	6	Walpole Park	P	W	6
Barnes Common	Co	C	5	Nunhead Cemetery	Ce	S	6	Berrylands	G	SW	5
Cannon Hill Common	Co	SW	5	Queensmill Road	G	C	6	Brompton Cemetery	Ce	C	5
Hanwell Cemetery	Ce	W	5	St. Andrew's Square	P	SW	6	Brooks Avenue	G	E	5

Table 4a continued

Princes Gardens	G	W	5	Walpole Park	P	W	6	Cannon Hill Common	Co	SW	5
Wanstead Flats	Co	E	5	Barnes Common	Co	C	5	Ealing Common	Co	W	5
West Ham Park	P	E	5	Brooks Avenue	G	E	5	Nunhead Cemetery	Ce	S	5
Brompton Cemetery	Ce	C	4	Princes Gardens	G	W	5	Sexby Gardens	P	S	5
Hampstead Heath	Co	N	4	Berrylands	G	SW	4	St. Andrew's Square	P	SW	5
Pymers Mead	G	S	4	Regent's Park	P	N	4	Regent's Park	P	N	4
Queensmill Road	G	C	4	Hampstead Heath	Co	N	3	Wanstead Flats	Co	E	3
St. Andrew's Square	P	SW	4	Highgate Cemetery	Ce	N	3	Hampstead Heath	Co	N	2
Brooks Avenue	G	E	3	St. Luke's Church	P	C	3	St. Luke's Church	P	C	2
St. Luke's Church	P	C	3	Wanstead Flats	Co	E	3	Princes Gardens	G	W	N/A

Table 4b. Plants species diversity at each transect, shown in descending order according to their floral diversity in all rounds combined.

All rounds combined Location Name	Habitat	Region	Total no. of plants
Sand's End	Wasteland	Central	33
Ferndale Street	Wasteland	East	33
Tolworth	Wasteland	South West	32
Ossary Road	Wasteland	South	28
Burgess Park	Common	South	25
Jenner Avenue	Wasteland	West	23
St. James' Lane	Garden	North	21
Sexby Gardens	Park	South	19
Woodgrange Park Cemetery	Cemetery	East	17
Tollington Road	Wasteland	North	17
Morden Cemetery	Cemetery	South West	16
Highgate Cemetery	Cemetery	North	16
Ealing Common	Common	West	16
Berrylands	Garden	South West	16
Nunhead Cemetery	Cemetery	South	15
Hanwell Cemetery	Cemetery	West	15
Brompton Cemetery	Cemetery	Central	15
West Ham Park	Park	East	14
St. Andrew's Square	Park	South West	14
Queensmill Road	Garden	Central	14
Barnes Common	Common	Central	14
Pymers Mead	Garden	South	13
Walpole Park	Park	West	12
Cannon Hill Common	Common	South West	12
Brooks Avenue	Garden	East	10
Wanstead Flats	Common	East	10
Princes Gardens	Garden	West	10
Regent's Park	Park	North	8
St. Luke's Church	Park	Central	7
Hampstead Heath	Common	North	7

Table 5. The top ten flowers most visited by workers of each bumble bee species over all of the rounds combined. The percentage of all observations accounted for by the top ten flowers are given for each species.

Bombus species	Plant species	No. of observations	*Bombus* species	Plant species	No. of observations	*Bombus* species	Plant species	No. of observations
B. terrestris	*Rubus fruticosus*	26	*B. pascuorum*	*Trifolium repens*	14	*B. hortorum*	*Deutzia* var	3
/ B. lucorum	*Trifolium repens*	20		*Cirsium arvense*	5		*Lonicera periclymenum*	3
	Cirsium vulgare	14		*Lamium album*	5		*Lamium album*	2
	Deutzia var	13		*Lathyrus latifolius*	5		*Bellis perennis*	2
	Sedum rupestre	12		*Bellis perennis*	4		*Ceratostigma willmottianum*	2
	Brassica napus	11		*Melilotus albus*	4		*Gentiana asclepiadea*	2
	Cirsium arvense	11		*Nepeta x faassenii*	4		*Ballota nigra*	1
	Geranium dissectum	10		*Galega officinalis*	4		*Trifolium pratense*	1
	Tagetes 'Tangerine Dream'	9		*Medicago sativa*	4		*Iris pseudacorus*	1
	Lathyrus odoratus	8		*Melilotus officinalis*	4		*Digitalis purpurea*	1
	% of all observations	43%		% of all observations	41%		% of all observations	90%
B. lapidarius	*Picris echioides*	15	*B. pratorum*	*Lamiastrum galeobdolon*	12	*B. humilis*	*Ballota nigra*	3
	Senecio jacobaea	5		*Rubus fruticosus*	5		*Lamium album*	1
	Lotus corniculatus	5		*Geranium pratense*	4		% of all observations	100%
	Erigeron 'Serenity'	5		*Hebe 'Bowles' variety*	3			
	Melilotus officinalis	4		*Lamium maculatum*	2			
	Picris hieracoides	4		*Geranium sanguineum*	2			
	Rubus fruticosus	3		*Staphylea pinnata*	2			
	Trifolium repens	2		*Trifolium repens*	1			
	Centurea nigra	2		*Rosa canina*	1			
	Centurea scabiosa	2		*Mysotis sylvatica*	1			
	% of all observations	64%		% of all observations	74%			

Table 6. The four flower species most 'preferred' and 'avoided' by workers of each bumble bee species over all of the rounds combined after the effect of plant species abundance is removed. The ratio between the total number of each species of *Bombus* observed and the total abundance of flowers that each species was observed foraging on was calculated. This ratio was used to calculate the expected number of bees recorded on a species of forage plant in relation to its abundance. A chi-squared test was applied to see if more or fewer bees were observed than expected. If there were more than expected then the plant was classed as 'preferred' and if there were fewer than expected it was classed as 'avoided'.

Bombus species	Plant species preferred	Plant species avoided
B. terrestris / *B. lucorum*	*Geranium dissectum*	*Penstemon* var.
	Philadelphus var.	*Potentilla fruticosa*
	Aruncus dioicus	*Pelargonium* var.
	Sedum rupestre	*Dahlia*
B. pascuorum	*Nepeta x faassenii*	*Penstemon* var.
	Lapsana communis	*Tagetes* 'Tangerine Dream'
	Solanum dulcamara	*Potentilla fruticosa*
	Galega officinalis	*Hypericum* 'Hidecote'
B. lapidarius	*Picris echioides*	*Penstemon* var.
	Erigeron 'Serenity'	*Tagetes* 'Tangerine *Dream'*
	Melilotus officinalis	*Potentilla fruticosa*
	Lotus corniculatus	*Hypericum* 'Hidecote'
B. pratorum	*Lamiastrum galeobdolon*	*Pelargonium* var.
	Hebe albicans	*Penstemon* var.
	Philadelphus var.	*Tagetes* 'Tangerine Dream'
	Geranium pratense	*Potentilla fruticosa*
B. hortorum	*Gentiana asclepiadea*	*Rubus fruticosus*
	Cerinthe major 'purpurascens'	*Pelargonium* var.
	Digitalis purpurea	*Penstemon* var.
	Lonicera periclymenum	*Tagetes* 'Tangerine Dream'
B. humilis	*Ballota nigra*	*Rubus fruticosus*
	Lamium album	*Pelargonium* var.
		Penstemon var.
		Tagetes 'Tangerine Dream'

Figure 1. A map of London to show the location of the study sites, their habitat and their geographical loocation.

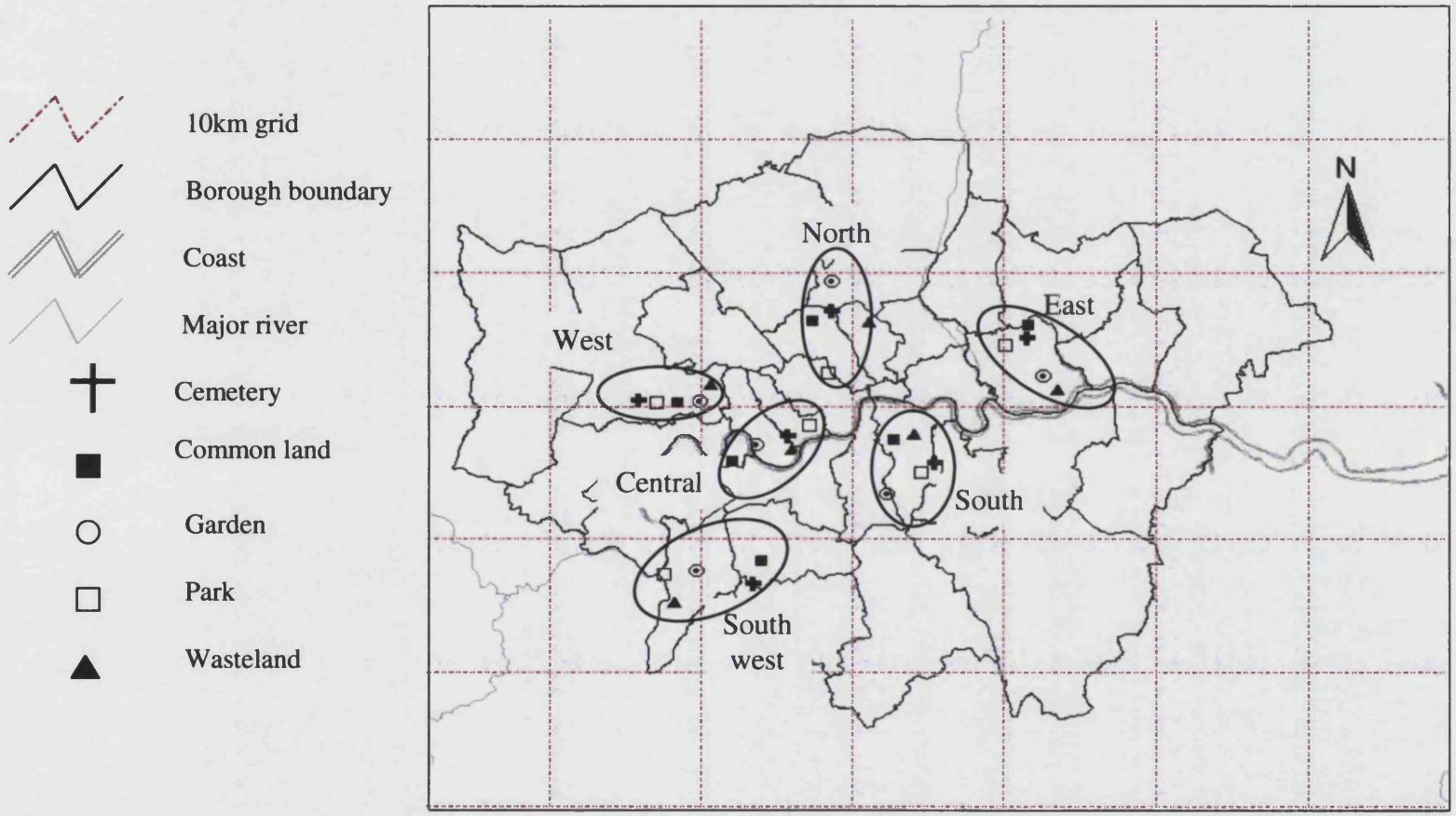


Figure 2. The phenology of each *Bombus* species and caste. Observations of *B. terrestris* and *B. lucorum* workers were combined for analysis due to their similarities in the field. Note the different scales on the y- axes. In the legends *W* denotes worker, *M* denotes male and *Q* denotes queen.

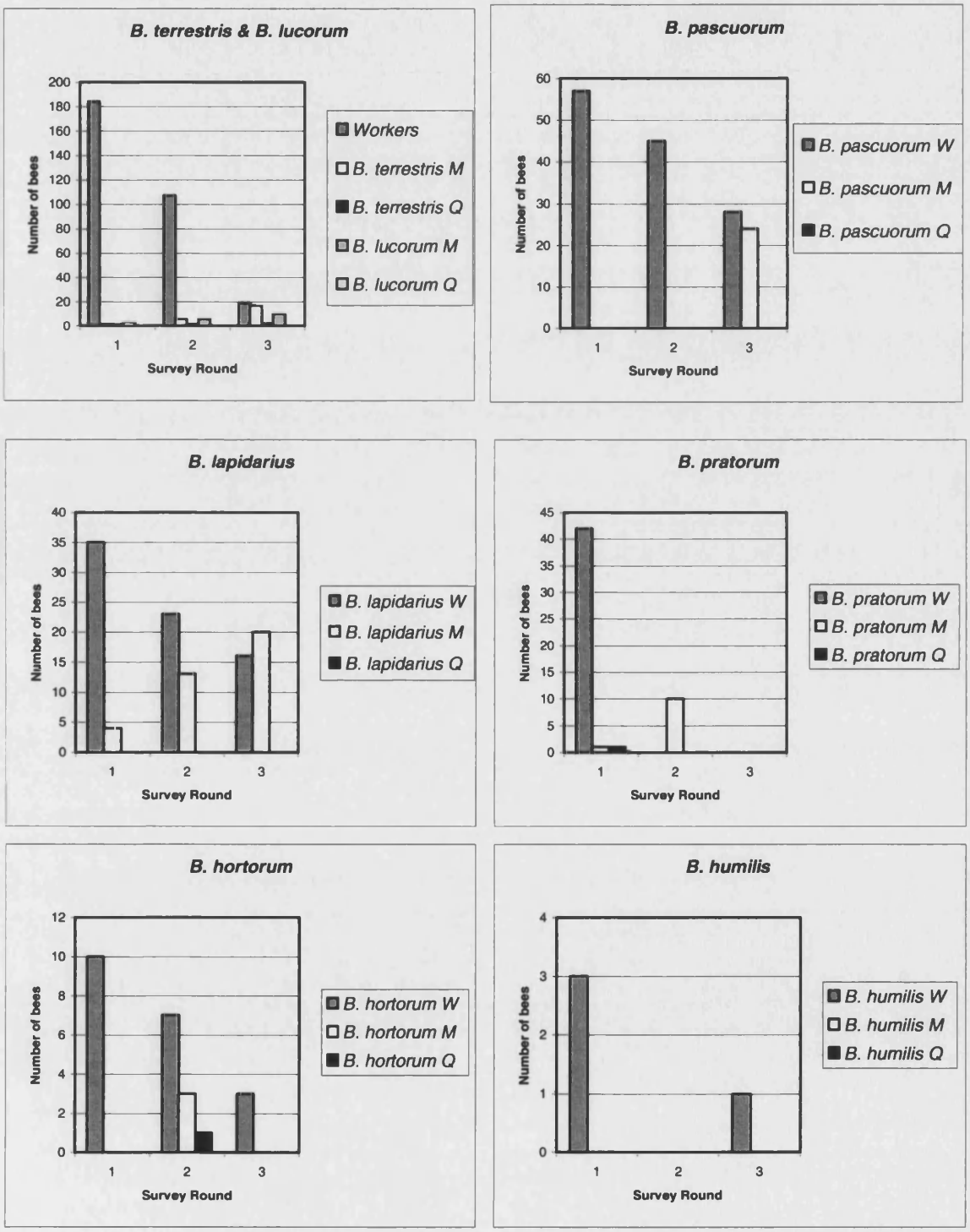


Figure 3. Percentage of *Bombus* species observations by region of London over all rounds combined. The six clusters of the five habitat types across London were named according to their geographical location. See Figure 1 for the locations of the regions.

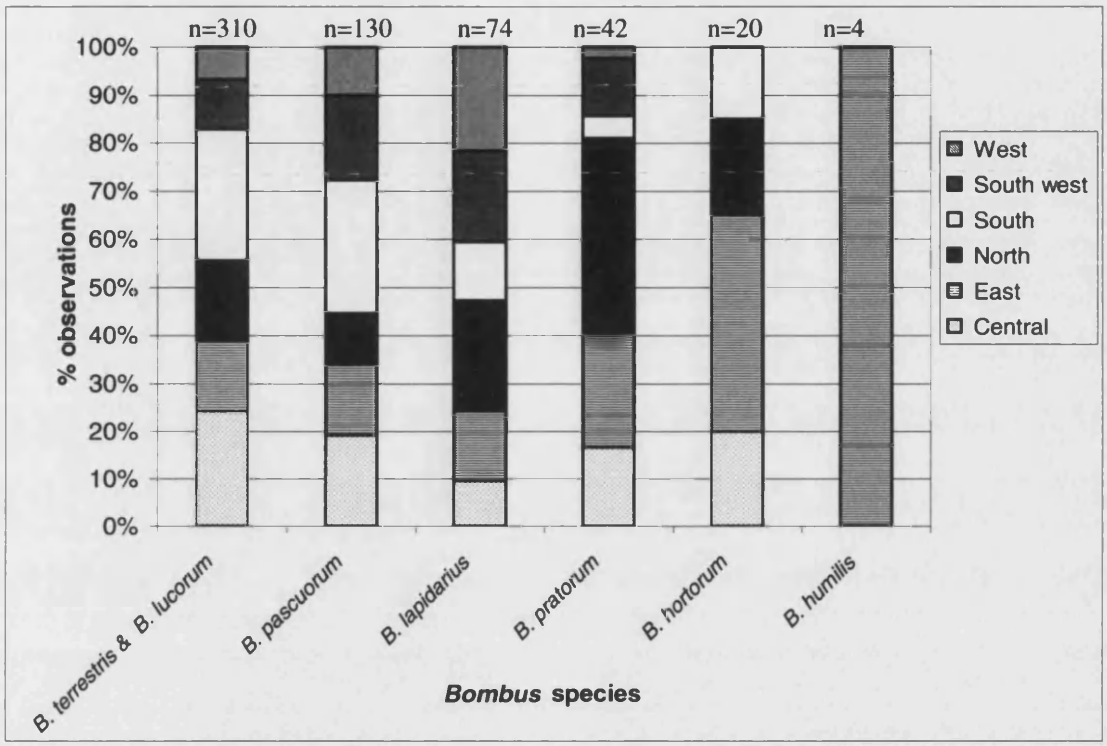


Figure 4. Percentage of *Bombus* species observations by habitat over all rounds combined.

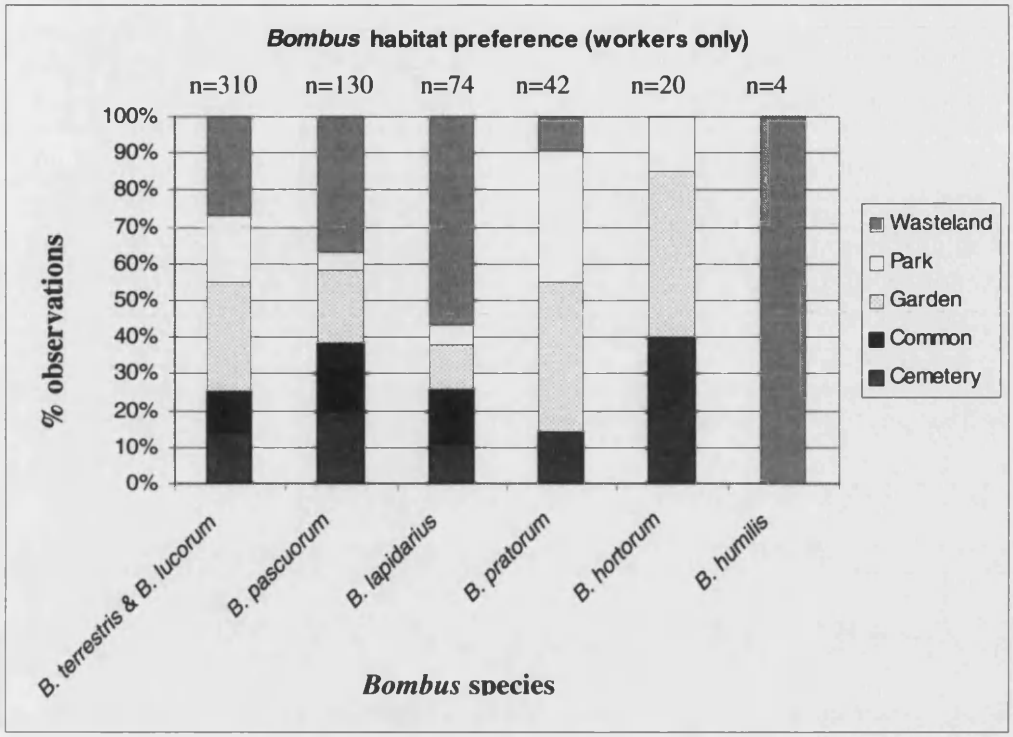


Figure 5. Floral abundance by habitat. Calculated as the percentage of quadrat squares that contained flowers. A total of 53,400 squares were sampled of which 15,715 squares were found to contain flowers.

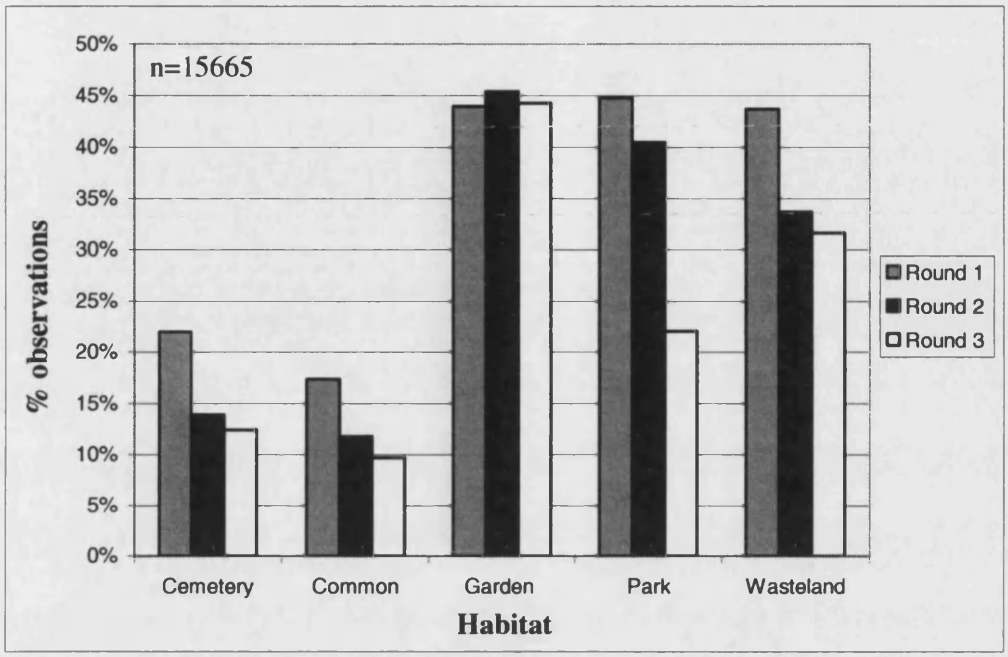


Figure 6. Observations of worker bumble bees by habitat shown as a percentage of all observations.

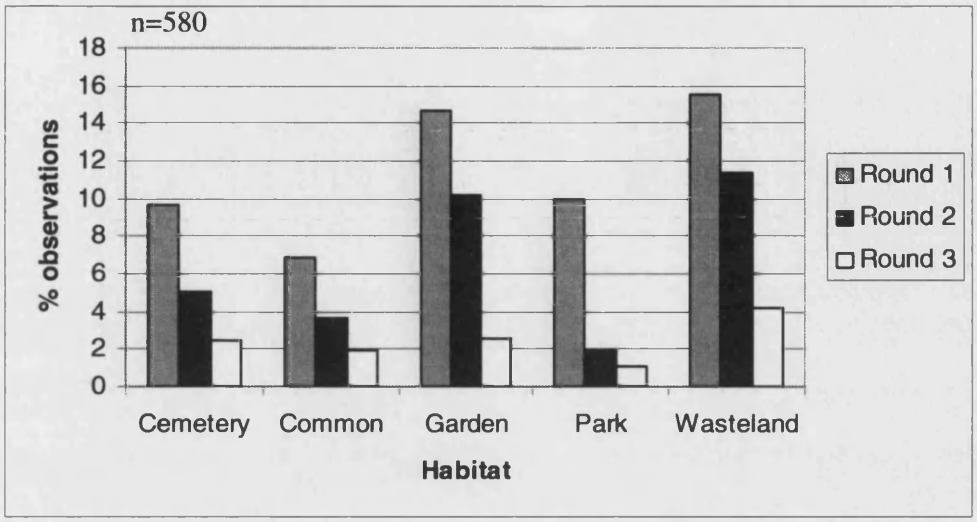


Figure 7. The proportion of native and non-native flower coverage recorded at each habitat.

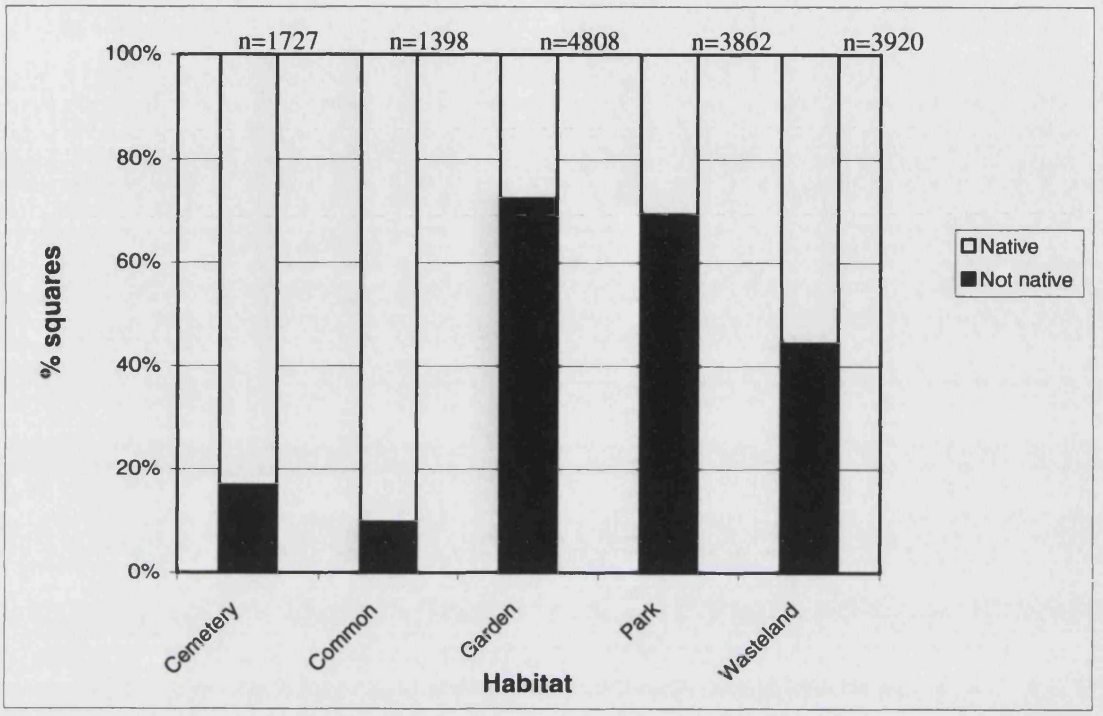
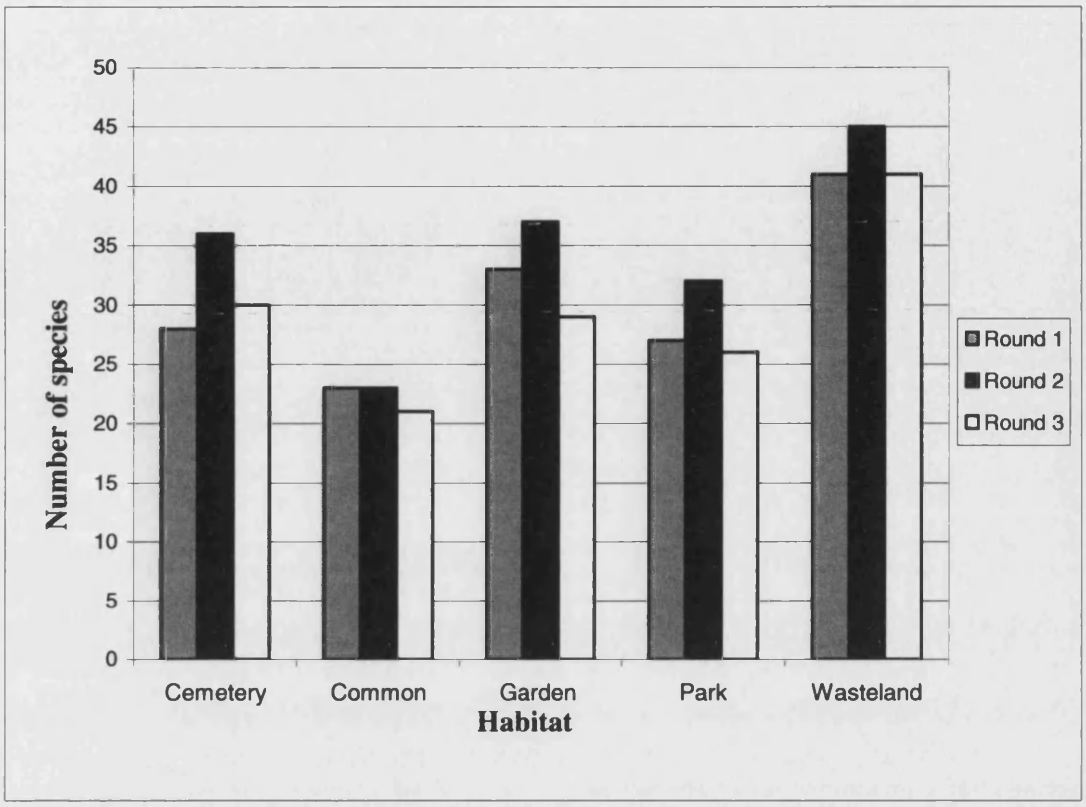


Figure 8. Floral species diversity by habitat.



Chapter 3

The distribution and foraging ecology of a rare bumble bee,

Bombus humilis, in a large urban area (London, U.K.).

SUMMARY

Bombus humilis is one of 19 species of non-parasitic British bumble bee and as a result of its severe decline in Britain in recent decades, it is one of five species to be given U.K. Biodiversity Action Plan (BAP) status (Anonymous, 1995, 1999). Prior to the present study, the main body of published work on this species came from a study conducted on unimproved grasslands (Carvell, 2001, 2002). In London, grasslands are scarce but *B. humilis* persists and sometimes thrives in some unexpected places. The overall aim of this study was to determine the status and foraging requirements of *B. humilis* in the urban environment of London, with the conservation of the species in mind. There were three specific goals. First, to establish the distribution of *B. humilis* in the London area. Second, to record the abundance of its castes over time. Third, to survey forage availability and identify the forage and habitat usage by *B. humilis* in this urban environment. The two sites where *B. humilis* was identified in the London-wide *Bombus* survey in 2000 (Chapter 2 of this thesis) formed the starting point for a systematic search for further sites in 2001. Other sites were found by chance. Potential sites were revisited throughout the spring and summer until the presence or absence *B. humilis* could be established. At two of these sites, four transects were established and surveyed nine times between 21st May and 19th August 2001. Foraging bumble bees were identified along transects and flowering plants were recorded using quadrats. Twelve sites were identified as supporting *B. humilis* throughout the flying season. The distribution of *B. humilis* followed the River Thames as far west as the Millennium Dome, and the River Lea as far north as the Walthamstow Marshes. Seven species of *Bombus* were identified during the study. *B. humilis* was the fourth most abundant species and made up 19% of all bumble bees observed. The number of *B. humilis* queens peaked in mid-June and the number of workers in late-July. The garden habitat

of the Thames Barrier Park was found to support the greatest number of foraging *B. humilis*. The majority of all observations of foraging *B. humilis* were on flowers in the Lamiaceae family, particularly the garden variety *Lavandula* x *intermedia* 'Dutch'. Species of plant native to the U.K. were not necessarily favoured forage of *B. humilis*. Finally, it is recommended that the profile of this species be raised so that the relevant bodies, at least, are aware of its presence. Known sites must be protected, nesting habitat identified and the species monitored for long-term population trends. Genetic work could be employed to address population and meta-population questions relating to long-term conservation management strategies. Future habitat development and enhancement schemes should look to the Thames Barrier Park as an excellent example of sympathetic development of valuable wasteland habitat. It is recommended that any wildflower areas are not mown until mid-September when they have set seed and the *B. humilis* nests have completed their cycle.

1. INTRODUCTION

Bombus humilis is one of 19 species of non-parasitic British bumble bee and, as a result of its severe decline in Britain in recent decades, it is one of five species to be given U.K. Biodiversity Action Plan (BAP) status (Anonymous, 1995, 1999). Despite being common and widespread in Continental Europe, *B. humilis* has never been recorded as abundant in England and Wales and there are no records of the species from Scotland or Northern Ireland. It is considered to be a 'southern local' species on account of its reaching its northern limit within Britain and southern Scandinavia (Williams, 1982). By the 1970s it had disappeared from its most northern sites and many inland sites, but still maintained a strong coastal distribution in the south, south-east, and south-west of England and parts of Wales. The decline continued and it currently exists as a number of isolated populations along the coast of southern England and Wales and at a few inland sites associated with the larger areas of chalk grasslands, including Salisbury Plain (Edwards, 2002b) (Figure 1). The decline of *B. humilis* and other bumble bee species has been attributed to the loss of their foraging, nesting and over-wintering habitats through modern agricultural practices. In particular, the mechanisation of hay-making and latterly the shift to silage making which requires nutrient enriched, 'weed'-free, grass to be cut early in the summer whilst still green, has led to the decline of wildflower meadows. For example, between 1932 and 1984 the total area of unimproved grassland in Britain decreased by 90% (Fuller, 1987). As a carder bee, *B. humilis* is a surface-nester and covers its nest with leaf and grass litter and moss. Hence, for nesting, it requires tall but open grassland vegetation with moss at the base of tussocks of grass (Edwards, 1998). Like many other species of *Bombus*, *B. humilis* is traditionally associated with flower-rich habitats that can supply it with a succession of forage throughout its flying season. As a longer-tongued bee (Medler, 1962), this

species is expected to favour flowers with longer corollas (Barrow & Pickard, 1984; Ranta & Lundberg, 1980; Teräs, 1976). Work carried out on Salisbury Plain in 1999 found that *B. humilis* preferred tall open grasslands rather than hard grazed flower-supporting areas and that there was a correlation between the abundances of *Trifolium pratense* and of *B. humilis* (Carvell, 2001, 2002). Native wild flowers from the Fabaceae family were identified as an especially important source of pollen and nectar for *B. humilis* (Goulson & Darvill, 2004) at the same study site. The habitats of Salisbury Plain and London are very different; in London grasslands and meadows are scarce but *B. humilis* persists in some unexpected places.

Although *B. humilis* is considered to be the least rare of the 5 U.K. BAP species, it is thought to be the only one that is still found in London in significant numbers and is therefore of particular interest to this study. As a U.K. BAP species occurring in London, *B. humilis* has a Local Biodiversity Action Plan dedicated to it, which has recently summarised local knowledge of the species (London Biodiversity Partnership, 2001a) with input from the present study. Prior to the present study, the knowledge of *B. humilis* distribution within the Thames Corridor had been based on data collected by several individuals and collated by the Bees, Wasps and Ants Recording Society (BWARS) (Edwards, 2002b). Most records were within 1km of the River Thames and the species was recorded as absent from some apparently suitable sites further inland. Within this Thames Corridor wasteland is highlighted as the habitat where *B. humilis* occurs most frequently and perennial, deep-corollaed members of the pea and dead-nettle families as their most frequently visited forage (London Biodiversity Partnership, 2001a).

Recent publications (Anonymous, 1999; London Biodiversity Partnership, 2001a) have highlighted the fact that very little is known about the ecology of *B. humilis* and it is this lack of knowledge, rather than lack of willing, that is hampering the development of a comprehensive Local Biodiversity Action Plan (London Biodiversity Partnership, 2001a) and thus conservation efforts on behalf of this species. The overall aim of this study was to establish the true status and forage requirements of *B. humilis* in London, with the purpose of contributing to its conservation management in the city. There were three specific goals. First, to establish the distribution of *B. humilis* in the London area. Second, to record the abundance of *B. humilis* and the succession of its castes over a single season. Third, to survey forage availability over time and investigate the forage and habitat usage of *B. humilis*. The nesting requirements of *B. humilis* were not explored due to time constraints. This study shows for the first time how this rare species interacts with and, in places, thrives in the urban environment.

2. MATERIALS AND METHODS

Between March and October 2001, I identified sites with the potential to support foraging *B. humilis* queens, workers and males. At each of two of these sites I established two 1m × 100m transects, along which forage availability and bumble bee species were surveyed. Each transect was surveyed approximately once a week.

Careful species identification of brown bumble bees was very important so as to eliminate confusion between the target species, *B. humilis,* the ubiquitous *B. pascuorum* and the rare *B. muscorum,* which historically has been recorded in London and is currently present in parts of the Thames Estuary (Edwards, 2002a; IBRA/BRC, 1980). Although *B. humilis* has a very distinctive brown band across the second abdominal tergite (T_2)(Prys-Jones & Corbet, 1991), colour variation can cause confusion. Voucher specimens were taken from each site and their identification confirmed with a hand lens and by dissecting out the sting sheath and viewing its distinctive shape under a dissecting microscope (Prys-Jones & Corbet, 1991).

2.1 Selection of study sites

B. humilis distribution

The London-wide bumble bee species survey carried out in 2000 (Chapter 2 in this thesis) detected *B. humilis* at a wasteland site in Ferndale Street, E6. *B. humilis* was also found in Woodgrange Park Cemetery (E7) in 2000, but not during the formal survey. These sites formed the starting point for the 2001 fieldwork, during which potential *B. humilis* sites in proximity to Ferndale Street and Woodgrange Park Cemetery were identified from an Ordnance Survey map and then visited. Other sites

were found by chance during many hours of footwork. If the site was accessible and forage was present then it was revisited throughout the spring and summer until the presence or absence of nest-searching or foraging *B. humilis* queens and workers could be established. The search for new *B. humilis* sites was continued in 2002 using the same method but no formal transects were conducted.

Forage availability and bumble bee species survey

Two of the sites, Temple Mills and Thames Barrier Park (Table 1; Figure 2), were selected as study sites for an investigation of the ecology and phenology of *B. humilis.* They were selected on the grounds of their accessibility and permanence and to incorporate maximum variation in habitat type and distance between two sites. They also appeared to support the greatest number of queens in the spring and so it was hoped they would also support many workers throughout the season. The Temple Mills stream transect runs along the overgrown path and bank of the Channelsea River, a tributary of the River Lea. The Temple Mills pond transect runs through Bully Point Nature Reserve which is an area of rough, but natural vegetation. The Lee Valley Regional Park Authority manages both of these sites. The Thames Barrier Park lies on the north side of the Thames Barrier and is managed by the London Development Agency. The wildflower transect runs through a 4-hectare seeded wildflower meadow. The garden transect runs along part of a 15m × 400m area of herbaceous and shrub borders.

2.2 Forage availability survey

Forage availability was surveyed at $1m^2$ quadrats placed at 15m intervals along each 1m × 100m transect. The quadrat locations were semi-permanently marked to ensure accurate replication over time. To sample the availability of potential forage I placed a $1m^2$ quadrat at 15m intervals along the 100m transect. The quadrat was sub-divided with string into one hundred $10cm^2$ units, known as squares from here on. Each square was surveyed for the presence or absence of inflorescences. Where inflorescences were present in a square they were identified to species and their presence was mapped by square onto a standardised data sheet. Using this method all of the plants in flower at the time, within the quadrat, were identified to species and the area they covered within the quadrat calculated. Wildflowers were identified using standard floras (Blamey & Grey-Wilson, 1989; Fitter *et al.*, 1996; Stace, 1997). Garden plants were identified as closely as possibly to variety (Brickell, 1996; Scott-Macnab, 1997).

2.3 Bumble bee species survey 'bee walk'

All transects were walked between 09:30 and 18:00, in dry weather and when temperatures were over 15°C. Five minutes were allowed to elapse between finishing the plant survey and starting the bee survey to enable the bees to settle back into their foraging pattern. The transect was taken at a slow, regular pace, and the time was noted at the start and finish of each transect. Each bee encountered was identified to species and caste and its activity recorded. Environmental variables were also noted and each transect was photographed on each visit. Each transect was walked once a week, or as close to once a week as weather permitted, from 9th May until 12th October 2001 (Table 1).

2.4 Statistical methods

All of the data were analysed using χ^2 tests. In many transects the total number of foraging observations on a plant species was low. So, in order to carry out the χ^2 tests presented in Table 6, the data had to be pooled in order to gain expected values greater than five. Before this was done the data were tested for homogeneity and where the χ^2 test proved not significant, i.e. the observations were distributed evenly, the data were pooled and subjected to further analysis. Where the expected values were still not greater than five, despite pooling, the observations that did not produce an expected value of more than five were pooled and classified as 'other'. All χ^2 tests were applied to the count data rather than percentage data. The abundance and phenology of all species and castes of bumble bees recorded were analysed. The analyses of habitat and forage usage were carried out for *B. humilis* only.

3. RESULTS

Twenty-nine days over 21 weeks were spent surveying the four transects. For the purpose of analysis the data were divided into weeks 1-21 (Table 1). For various reasons, for example poor weather, all four transects were not visited every week. Only those weeks where all four transects were surveyed in the same week are included in this analysis, unless stated otherwise. This amounted to nine weeks. The wildflower meadow at Thames Barrier Park was mown on 13th August, after which the transect could no longer be surveyed.

3.1 Bumble bees

B. humilis distribution

The current known distribution of *B. humilis* in the London area is shown (Figure 3) and combines previous knowledge collated by Peter Harvey of BWARS with sites recorded during this study. In addition to the two sites identified in 2000, seven sites in 2001 and a further three sites in 2002 were found to support foraging *B. humilis* (Table 2). The distribution of *B. humilis* was found to be generally riverine, occupying the north and south sides of the River Thames and extending as far west as the Millennium Dome on the south side. The River Lea Valley marks the western extent of the range on the north side of the River Thames and provides a corridor to the Hackney Marshes, eight kilometres north of the River Thames.

During the search for *B. humilis* sites two more species of bumble bee, *B. sylvarum* and *B. ruderarius,* were found in central London, taking the total number of species found in London in the present study to nine. A single *B. sylvarum* queen was caught on 31/05/01at the Thames Barrier Park site and identified, photographed and released.

The following week two *B. sylvarum* queens were observed, one dead and one alive. The dead one was collected and is held in my reference collection. A total of four *B. sylvarum* workers were observed through July and August and two males in September. *B. ruderarius* workers were identified at the Millennium Village (SE10) through August but also at very low densities. Their locations from this study combined with data collated by Peter Harvey of BWARS are shown (Figure 4).

Bumble bee species overall abundance

During the study period 2, 207 foraging bumble bees (workers, queens and males) were recorded (Table 3). There was a significant difference in the relative abundance of species in all weeks combined (χ^2 = 1209.5, d.f. = 6, P < 0.001). The following species were identified in order of overall worker abundance: *B. terrestris / B. lucorum* (41%), *B. pascuorum* (21%), *B. humilis* (19%), *B. lapidarius* (10%), *B. pratorum* (5%), *B. hortorum* (4%).

Bumble bee phenology

The first *B. humilis* queen was observed on 9th April at the Beckton Alps (E6). The Thames Barrier and Temple Mills sites and transects were only identified at the end of May and *B. humilis* queens were already present. Of queens of all seven species, *B. humilis* queens were the last to peak in abundance, in the first week of June. Along the transects the relative abundance of workers of each species varied over the weeks (Figure 5). In weeks 3, 5 and 6, *B. pratorum* workers were significantly more abundant than workers of any other species (week 3: χ^2 = 153.0, d.f. = 6, P < 0.001; week 5: χ^2 = 34.6, d.f. = 6, P < 0.001; week 6: χ^2 = 35.4, d.f. = 6, P < 0.001) (Figure 5). *B.*

pascuorum was the most abundant species in week 8 (χ^2 = 11.9, d.f. = 6, P < 0.1) and in weeks 10 and 12 *B. terrestris* / *B. lucorum* were the most common (week 10: χ^2 = 72.6, d.f. = 6, P < 0.001; week 12: χ^2 = 139.8, d.f. = 6, P < 0.001) (Figure 5). By week 13, *B. humilis* was the most abundant species (χ^2 = 175.8, d.f. = 6, P < 0.001) (Figure 5). Finally, in week 15, *B. pascuorum* was the most abundant (χ^2 = 295.6, d.f. = 6, P < 0.001) (Figure 5). *B. humilis* workers, males and queens were still observed on 29th September in week 21 and workers and males were even observed, in very low numbers, on 13th October.

B. humilis abundance by transect

Overall, combining sexes and castes, across the four transects, significantly more *B. humilis* were recorded along the Thames Barrier garden transect (χ^2 = 322.3, d.f. = 3, *P* < 0.001), followed by the Thames Barrier wildflower transect (Figure 6). Significantly more *B. humilis* queens and workers were identified along the garden transect, but males visited no transect more than the others (workers: χ^2 = 238.5, d.f. = 3, *P* < 0.001; queens: χ^2 = 80.9, d.f. = 3, *P* < 0.001; males: χ^2 = 5.9, d.f. = 3, P > 0.05).

3.2 Plants

Flowering plant abundance

Overall during the study period, 58% of all of the area surveyed contained flowers. The Thames Barrier garden transect provided a significantly greater amount of potential forage than the other three transects (χ^2 = 331, d.f. = 3, *P* < 0.001). The Thames Barrier garden transect provided the most potential forage in all of the weeks, except for weeks 8, 10 and 11 when significantly more forage was recorded along the Thames Barrier

wildflower transect (week 3: $\chi^2 = 178.3$, d.f. = 3, $P < 0.001$; week 5: $\chi^2 = 89.7$, d.f. = 3, $P < 0.001$; week 6: $\chi^2 = 113.0$, d.f. = 3, $P < 0.001$; week 8: $\chi^2 = 65.8$, d.f. = 3, $P < 0.001$; week 10: $\chi^2 = 59.5$, d.f. = 3, $P < 0.001$; week 11: $\chi^2 = 36.0$, d.f. = 3, $P < 0.001$; week 12: $\chi^2 = 47.4$, d.f. = 3, $P < 0.001$; week 13: $\chi^2 = 25.0$, d.f. = 3, $P < 0.001$; week 15: $\chi^2 = 57.5$, d.f. = 3, $P < 0.001$), (Figure 7). Over all of the transects, there was significantly more forage available in week 5 than in any other week ($\chi^2 = 301.0$, d.f. = 8, $P < 0.001$). There was variation in the timing of the peak in abundance of forage between the sites. At the Thames Barrier garden transect the greatest abundance of forage was recorded in week 6 ($\chi^2 = 224.0$, d.f. = 8, $P < 0.001$), but at the Temple Mills stream transect abundance peaked in week 5 ($\chi^2 = 144.0$, d.f. = 8, $P < 0.001$). Forage abundance peaked in week 6 at the Thames Barrier wildflower transect ($\chi^2 = 224.1$, d.f. = 8, $P < 0.001$) and in week 12 at the Temple Mills pond site ($\chi^2 = 110.8$, d.f. = 8, $P < 0.001$).

The most abundant species of flower recorded was *Galega officinalis* (Goat's Rue) contributing to 9% of all squares sampled, closely followed by *Rubus fruticosus* (7%). Twenty six percent of all squares surveyed contained flowers from the Fabaceae family and 22% contained flowers from the Lamiaceae. Across all four of the transects, 59% of the flowers were native to the U.K., and there was a significant difference in the abundance of native and non-native flowers across the transects ($\chi^2 = 7033.3$, d.f. = 1, $P < 0.001$), with a dominance of native flowers at the Temple Mills pond and stream transects (pond: $\chi^2 = 6068.4$, d.f. = 1, $P < 0.001$; stream: $\chi^2 = 5643.8$, d.f. = 1, $P < 0.001$). Non-native flowers were most abundant at the Thames Barrier garden and wildflower transects (garden: $\chi^2 = 7091.7$, d.f. = 1, $P < 0.001$; wildflowers: $\chi^2 =$

7190.0, d.f. = 1, $P < 0.001$). There was a significantly greater abundance of perennial flowers compared with annuals, biennials or shrubs ($\chi^2 = 12614.0$, d.f. = 3, $P < 0.001$) across all of the transects and within the transects (Temple Mills pond: $\chi^2 = 3240.3$, d.f. = 3, $P < 0.001$; Temple Mills stream: $\chi^2 = 2103.6$, d.f. = 3, $P < 0.001$; Thames Barrier garden: $\chi^2 = 8912.3$, d.f. = 3, $P < 0.001$; Thames Barrier wildflowers: $\chi^2 = 3365.2$, d.f. = 3, $P < 0.001$).

Flowering plant diversity

During the plant survey 101 species from 23 families were identified in flower. The Temple Mills pond transect was found to support twenty six species of plants in flower at some point in the study, and the Temple Mills stream transect and Thames Barrier garden transect both had 17 species each. Twenty species were identified from the Thames Barrier wildflower site. There was, however, no significant difference in the floral species diversity between the sites overall ($\chi^2 = 2.01$, d.f. = 3, $P > 0.05$). There was no significant difference in the floral species diversity between the weeks overall ($\chi^2 = 1.2$, d.f. = 8, $P > 0.99$).

B. humilis forage utilisation

Pooling all three castes, *B. humilis* was observed foraging on 55 species or varieties of flowers from 14 families (Table 4). The majority of foraging *B. humilis* were recorded on flowers from the Lamiaceae family (Figures 8, 9). Workers and queens did not necessarily visit different forage plant families according to their abundance (workers: $\chi^2 = 682.6$, d.f. = 22, $P < 0.001$; queens: $\chi^2 = 35.6$, d.f. = 4, $P < 0.001$). In relation to its low abundance, the Lythraceae family attracted significantly more *B. humilis*

workers and queens than any other family (workers: χ^2 = 266.0 d.f. = 8, $P < 0.001$; queens: χ^2 = 67.5 d.f. = 6, $P < 0.001$).

Across all the transects combined, *B. humilis* workers were found foraging on 38 species from 10 families. The greatest proportion of *B. humilis* workers (27%) was recorded foraging on *Lavandula* x *intermedia* 'Dutch'. Other species favoured by *B. humilis* workers were *Trifolium pratense* (Red clover), *Lotus corniculatus* (Common bird's-foot-trefoil) and *Perovskia atriplicipifolia* 'Blue Spire'. Despite being recorded in only 1.5 % of squares, *Lythrum virgatum* 'Rose Queen' attracted 10.4% of foraging workers, seven times more than expected. Common and abundant plant species that attracted no *B. humilis* workers were *Rubus fruticosus* (Blackberry) and *Geranium endressii* 'Wargrave pink.' Queens were observed foraging on 15 species from 7 families, the greatest proportion of which (13%) were recorded foraging on *Nepeta sibirica* 'Six Hills Giant' (Cat mint). Spring queens frequently visited *Salvia pratensis* (Meadow clary) and *Salvia officinalis* (Common sage) and new queens exploited a wide range of flowers, including *Vicia sativa* (Common vetch) and *Lamium album* (White dead-nettle). *B. humilis* males were observed foraging on 10 species from 10 families. The greatest proportion of males (31%) was observed on *Perovskia atriplicipifolia* 'Blue Spire' and *Lavandula* x *intermedia* 'Dutch', whilst *Galega officinalis* and *Rubus fruticosus* were avoided.

Significantly more *B. humilis* workers and queens were observed foraging on non-native species than on native species but males showed no significant bias (workers: χ^2 = 68.5 d.f. = 1, $P < 0.001$; queens: χ^2 = 8.2 d.f. = 1, $P < 0.001$; males: χ^2 = 2.3 d.f. = 1,

$P > 0.05$). Both worker and male *B. humilis* visited shrubs more frequently than annual, biennial or perennial flowers (workers: $\chi^2 = 153.0$ d.f. = 3, $P < 0.001$; males: $\chi^2 = 24.0$ d.f. = 3, $P < 0.001$), whereas queens utilised perennial flowers more frequently (queens: $\chi^2 = 34.6$ d.f. = 3, $P < 0.001$).

4. DISCUSSION

B. humilis distribution

The strip of derelict Thames-side industrial land on the eastern side of London is clearly being utilised by *B. humilis* as a corridor between the Thames Estuary and London (Figure 3). Similarly the valley of the Lea River links the River Thames with flower-rich patches further inland to the north. It is highly likely that, with time and effort, this species will be found at locations further north than the Essex and Middlesex Filter Beds. It cannot be ascertained from this study whether the bees recorded in this study form an independent, self-sustaining, London population or whether the population relies on being replenished by a continuous overspill of queens from the Thames Estuary population, approximately 40 kilometres to the east of the Thames Barrier. Genetic studies adapted from those described in Chapter 5 in this thesis (Chapman *et al.*, 2003) would be very revealing in this context, particularly for investigating the effects of deleting patches from an already narrow habitat matrix. Now that a technique has been developed for the non-lethal sampling of DNA from bumble bees (Holehouse *et al.*, 2003), genetic studies can be applied to rare species with a clear conscience.

Both *B. ruderarius* and *B. sylvarum* are rarer than *B. humilis* and *B. sylvarum* has been noted as being in danger of becoming extinct (Edwards & Telfer, 2001). Due to their paucity, very little can be concluded from the observations of these two species made in the present study, but in the same way that the 2000 field season provided a starting point for the *B. humilis* work so too could these observations provide the starting point for further studies. Harvey (2001) compared the Canvey Northwick site, in the Thames Estuary, approximately 35 kilometres east of the Thames Barrier, with those in other reports and concluded that it may support the most dense reproductive population of *B. sylvarum* in the U.K..

Bumble bee species overall abundance

In the 2000 London-wide bumble bee species survey, *B. humilis* was the least abundant species, representing under 1% of all workers observed. In the 2001 survey, which focused on the habitat frequented by *B. humilis*, its relative abundance increased such that it was found to be the fourth most abundant species, mainly at the expense of *B. terrestris* / *B. lucorum*, which still represented the most abundant species but which occurred at a frequency 12% less than their London-wide average. *B. pascuorum*, *B. lapidarius*, *B. pratorum* and *B. hortorum* occurred at frequencies all within 3% of their London-wide average. During a five week-long study of a 4-km^2 part of Salisbury Plain Training Area, Carvell (2002) identified 74 *B. humilis* bees. In the present nine week-long study of four 100m^2 transects, 478 *B. humilis* bees were recorded. Although the survey methods used in the two studies are not statistically comparable, it is clear that the numbers of *B. humilis* recorded in London in the present study are substantial.

Bumble bee phenology

The phenology described in this study generally concurs with that of other observations (Benton, 2000; Goodwin, 1992, 1995; Prys-Jones & Corbet, 1991) and is in greater detail than that described in Chapter 2 of this thesis (though based on a narrower selection of sites). *B. pratorum* workers peak in number first, in early June, followed by *B. hortorum* in early July. *B. terrestris* / *B. lucorum* and *B. lapidarius* have very similar phenologies, with the first workers appearing in late May, gradually building to a peak of abundance in early August and slowly declining in abundance until the last week of September. Their workers also have the longest flying season of all of the species in this study. *B. pascuorum* has a long season with worker numbers increasing steadily between early June and peaking in mid-August before tailing off by the end of September. The workers of *B. humilis* were the last to appear, being observed for the first time in the first week of July and rapidly building up to a peak of abundance by the end of July and the first week of August. The post-peak decline in *B. humilis* worker numbers is rapid, but workers, males and queens were still recorded in the last week of September.

B. humilis abundance by transect

Given that the Thames Barrier wildflower meadow was composed of predominantly native species and that 71% of available forage was from the deep-corollaed Fabaceae family, it was surprising that *B. humilis* did not favour this habitat over the garden, as in a recent study on Salisbury Plain *B. humilis* was shown to favour this family above all others (Goulson & Darvill, 2004). The Thames Barrier garden transect, which provided the greatest abundance (and therefore density), of forage throughout the study period, also supported the greatest number of *B. humilis* workers and queens. Many of the species and 52% of the forage plants recorded along the garden transect are from the

family Lamiaceae, for example *Lavandula* x *intermedia* 'Dutch', *Salvia pratensis* (meadow clary) and *Nepeta* 'Six Hills Giant' (cat mint). The Temple Mills transects run through a much more natural environment with an impressive natural diversity of native flora but without the artificially high densities of flowers. Although the numbers of *B. humilis* recorded at these transects were one fifth of those recorded at the Thames Barrier, Temple Mills is more similar to the other sites where *B. humilis* was identified during this study. The less intensive maintenance regime at Temple Mills produced a large area of undisturbed rough grassland, which is home to a notable range of invertebrates including the Nationally Scarce Roesel's bush-cricket (*Merioptera roeselli*). Although no attempts were made to search the area, this grassland appears to be ideal nesting habitat for *B. humilis.*

Flowering plant abundance

In the 2000 London-wide survey described in Chapter 2 of this thesis, 29% of squares surveyed contained plants in flower. In the present study, this figure was 57.5% overall (50% at the Temple Mills sites and 65% at the Thames Barrier sites). The herbaceous and shrub borders of the Thames Barrier Park were designed to give the area intense and continuous colour throughout the spring and summer months; therefore it is not surprising that the Thames Barrier garden transect provided the overall greatest abundance of potential forage during the study. For example, the thick banks of *Lavandula* x *intermedia* 'Dutch' provided a substantial source of forage for 11 of the 21 weeks of this study. This was only interrupted from the last week of June to mid-July when the peak in flower abundance in the wildflower area coincided with the trough in flower abundance in the garden area. The Temple Mills stream and pond transects were the most natural of the four transects so did not support such a high

abundance of forage as the Thames Barrier transects. Interestingly, the peak in overall forage abundance occurred in the first week of June (week 5). The importance of the existence of a mosaic of habitats is highlighted by the fact that not every site exhibited peaks and troughs in its floral abundance at the same time, so bumble bees always had somewhere to forage (Figure 7).

Flowering plant diversity

Although the Temple Mills pond transect only provided the third greatest abundance of forage, it did support the greatest plant species diversity, with six more species than the sown wildflower meadow at the Thames Barrier Park. The site in which the Temple Mills pond transect was situated is managed as a nature reserve and the more invasive species present in the area, such as *Impatiens glandulifera* (Himalayan balsam) and *Fallopia polygonum* (Japanese knotweed), have been deliberately excluded. *Rubus fruticosus* and other 'weeds' such as nettles were also kept under control to restrain succession and allow the perennial wildflowers, so favoured by bumble bees and other pollinators, to thrive.

B. humilis forage utilisation

Although London does not contain the pristine, unimproved, flower-rich habitat favoured by *B. humilis,* in places it clearly supports the forage abundance and diversity required to sustain healthy populations. In fact, 66% of *B. humilis*' forage species on Salisbury Plain (Carvell, 2002) were also recorded in this study. For example, the native wildflowers *Trifolium pratense* and *Lotus corniculatus* were abundant, and *Vicia cracca* and *Echium vulgare* were present, at three of the four transects. The Thames Barrier transects had an exceptionally high abundance of available forage for all of the

species of bumble bee, but especially for *B. humilis*, which is known to often forage on species from the Lamiaceae and Fabaceae families (Benton, 2000).

Not only did *Lavandula* x *intermedia* 'Dutch' attract the largest number of *B. humilis* workers, it also attracted more than was expected, given its abundance. The same applies to *Perovskia atriplicipifolia* 'Blue Spire' and *Lythrum virgatum* 'Rose Queen'. Given this, it is not surprising to learn that *B. humilis* did not visit native species more than non-native species in this garden habitat. It is encouraging to find *B. humilis* foraging on these species, as they are common and attractive garden plants. The forage list accumulated (Table 4) substantially adds to the range of forage plants provided by Benton (2000), which was compiled from observations on unimproved grassland sites. *B. humilis* may not forage on such a broad range of species as *B. pascuorum*, for example, but the fact that the forage list (Table 4) contains 66 species of forage plant, many of which are common wildflowers or garden plants, means that forage can be provided for *B. humilis* relatively simply.

The survey method employed in this chapter is very similar to that described in Chapter 2. Therefore, refer to the discussion section of Chapter 2 for its shortcomings and limitations.

Recommendations

The single greatest threat to *B. humilis* is the loss of its habitat and in the urban environment this is in the form of the loss of wasteland sites to development. Even during one field season several sites, including the clover-rich Beckton Alps, were developed, and Woodgrange Park Cemetery was 'tidied up'. This process involved the

cutting of grass and flowering plants and the removal of many of the larger flowering shrubs resulting in the loss of forage and undisturbed nesting sites. With proposed plans for the National Aquarium, the Olympic Village and hundreds of new homes in the lower Lea Valley and Silvertown areas, it seems that the most likely outcome will be for the present continuous, but narrow, matrix of suitable habitat to become seriously fragmented. This will result in *B. humilis* and probably other species being rapidly squeezed eastwards out of urban London, unless a carefully managed compromise can be achieved. Despite its B.A.P. status, *B. humilis* does not have any legal protection; however, Sites of Special Scientific Interest (SSSI) or Sites of Importance for Nature Conservation (SINCs) do. As nationally and regionally scarce species, *B. humilis*, *B. sylvarum* and *B. ruderarius* should make it more likely that potential sites receive SSSI or SINC designation. The profile of *B. humilis* must be raised so that at least the relevant site managers and Wildlife Trusts are aware of its presence. Current sites must be protected, marginal sites enhanced and new sites developed using current good sites as models. All known sites must be monitored for long-term population trends. This would make an excellent long-term study for biology undergraduates or M.Sc. students to adopt. Similarly, genetic work could be employed to address population questions relating to long-term conservation management strategies.

In an ideal world a continuous matrix of wastelands would be given legal protection and preserved for a range of threatened urban species such as *B. humilis*, the Black Redstart (*Phoenicurus ochruros*) and the Common Lizard (*Lacerta vivipara*) (London Biodiversity Partnership, 2001b). If social and economic pressures are so strong that the preservation of sufficient wastelands cannot be justified then an alternative must be sought. There is no doubt that the Thames Barrier Park site is an exceptional site, not

just for the East End, but for the whole of London. Covering 8.9 hectares and costing £13 million to develop, this area was the site of a chemical factory between 1869 and 1969. This site should therefore be looked to as an almost perfect example of how to satisfy the requirements of a housing development, a public park and a haven for rare bumble bees simultaneously.

Although *B. humilis* nests were not deliberately sought during this study, none were found even by chance. Finding and protecting areas that are likely to harbour nests is a priority. Within the lower Lea Valley and Silvertown areas there are several sites to which it was not possible for me to gain access. If a longer-term study in this species in this area should be undertaken, then it would be worth gaining access to them to look for nests.

In the summer of 2001 the wildflower meadow at the Thames Barrier Park was mown in mid-August, once the dominant *Galega officinalis* had finished flowering and become unsightly. This was unfortunate timing as even the species still in flower were removed before *B. humilis* worker numbers had started to decline. It is recommended that wildflower sites are not mown until they have set seed. This would allow any nests to complete their cycle and ensure that any late flowering species that this late-foraging bumble bee species may rely on are not removed prematurely. Although the area may look untidy for several weeks a public information sign outlining the reason for it would not only reduce any criticism of the site managers but also draw the public's attention to the rarity on their doorstep.

Table 1. The dates on which each transect was surveyed are marked *. The Thames Barrier wildflower meadow was mown on 13th August, after which it could no longer be surveyed.

Week	Date (2001)	Transect name			
		Temple Mills Pond	Temple Mills Stream	Thames Barrier Garden	Thames Barrier Wildflowers
1	7 May – 13 May	*	*		
2	14 May - 20 May	*	*		
3	21 May - 27 May	*	*	*	*
4	28 May - 3 June	*	*		
5	4 June - 10 June	*	*	*	*
6	11 June - 17 June	*	*	*	*
7	18 June - 24 June			*	*
8	25 June - 1 July	*	*	*	*
9	2 July - 8 July			*	*
10	9 July - 15 July	*	*	*	*
11	16 July - 22 July	*	*	*	*
12	23 July - 29 July	*	*	*	*
13	30 July - 5 August	*	*	*	*
14	6 August - 12 August			*	*
15	13 August - 19 August	*	*	*	*
16	20 August - 26 August	*	*	*	
17	27 August – 2 September	*	*	*	
18	3 September - 9 September				
19	10 September - 16 September				
20	17 September - 23 September				
21	24 September - 30 September	*	*	*	
22	1 October – 7 October				
23	8 October – 14 October			*	

Table 2. Site names and locations where *B. humilis, B. sylvarum* and *B. ruderarius* were found in the years 2000-2002. *Q = queen, W = worker, M = male. Two sites were identified in 2000 during the London-wide bumble bee survey (Chapter 2 of this thesis). A further seven sites were identified in 2001 as described in the text. Where possible known *B. humilis* sites were revisited in 2002 to see if they were still suitable habitats for *B. humilis* and to see if *B. humilis* was still present. This search yielded a further three sites.

Site Name	Species	*Caste	Grid Reference	Years searched	Years observed	Habitat Type
Thames Barrier Park (E16)	*B. humilis*	Q, W, M	TQ412800	2001, 2002	2001, 2002	Public garden / wildflower meadow / formal garden
Thames Barrier Park (E16)	*B. sylvarum*	Q, W, M	TQ412800	2001, 2002	2001, 2002	Public garden / wildflower meadow / formal garden
Maryon Park (SE18)	*B. humilis*	Q, W, M	TQ418787	2002	2002	Grassy park rich in labiates
Beckton Alps (E6)	*B. humilis*	Q, W, M	TQ431820	2001, 2002	2001, 2002	Dry ski slope fringed with dense areas of Fabaceae
Ferndale Street (E6)	*B. humilis*	Q, W, M	TQ435814	2000, 2001, 2002	2000, 2001, 2002	Wasteland
Mary Magdalene Cemetery (E6)	*B. humilis*	Q, W, M	TQ429823	2001, 2002	2001, 2002	Low maintenance cemetery
Woodgrange Park Cemetery (E7)	*B. humilis*	Q, W	TQ418852	2000, 2001	2000	Low maintenance cemetery
Bully Point Nature Reserve (E15)	*B. humilis*	Q, W, M	TQ375847	2001, 2002	2001, 2002	Managed nature reserve
Temple Mills Cycle Circuit (E15)	*B. humilis*	Q, W, M	TQ377851	2001, 2002	2001, 2002	Grassy park
Hackney Marshes (E5)	*B. humilis*	Q, W	TQ360866	2002	2002	Banks of River Lea, Essex & Middlesex Filter Beds.
Sorrell Lane (E14)	*B. humilis*	Q	TQ388810	2001, 2002	2001, 2002	Wasteland
Millennium Village (SE10)	*B. humilis*	Q, W, M	TQ398795	2001, 2002	2001, 2002	Grassy park / wildflower meadow / nature reserve
Millennium Village (SE10)	*B. ruderarius*	W, M	TQ398795	2002	2002	Grassy park / wildflower meadow / nature reserve
Ruston Road (SE18)	*B. humilis*	Q	TQ422792	2002	2002	Wasteland site

Table 3. Summary of the number of bumble bees, by species and caste, identified foraging in each week of the study between 21st May and 19th August 2001.

	Week number									
Species / Caste	3	5	6	8	10	11	12	13	15	**Total**
Worker										
B. terrestris / B. lucorum	7	13	24	34	84	100	157	151	156	726
B. pascuorum	1	0	0	21	34	38	56	80	147	377
B. lapidarius	4	9	10	15	14	17	29	38	32	168
B. pratorum	38	17	19	10	3	0	0	3	1	91
B. hortorum	1	2	5	13	14	18	12	10	1	76
B. humilis	0	1	0	5	8	17	74	114	119	338
Total worker	**51**	**42**	**58**	**98**	**157**	**190**	**328**	**396**	**456**	**1776**
Male										
B. terrestris	0	0	2	2	3	0	2	2	9	20
B. lucorum	0	0	0	0	0	0	0	2	13	15
B. pascuorum	0	0	0	0	2	1	3	6	9	21
B. lapidarius	0	0	0	0	0	0	2	2	4	8
B. pratorum	1	1	0	6	12	14	5	4	3	46
B. hortorum	0	0	0	5	3	4	5	7	10	34
B. humilis	0	0	0	0	0	0	0	5	16	21
Total male	**1**	**1**	**2**	**13**	**20**	**19**	**17**	**28**	**64**	**165**
Queen										
B. terrestris	22	4	1	0	0	1	0	1	1	30
B. lucorum	7	2	0	0	0	0	1	0	0	10
B. pascuorum	28	8	7	1	0	0	1	0	0	45
B. lapidarius	11	6	0	0	0	0	0	0	1	18
B. pratorum	7	3	0	1	0	0	2	1	0	14
B. hortorum	6	10	7	2	1	1	0	0	3	30
B. humilis	14	31	40	28	1	1	2	2	0	119
Total queen	**95**	**64**	**55**	**32**	**2**	**3**	**6**	**4**	**5**	**266**

Table 4. A complete list of *B. humilis* forage plants accumulated over the 2000, 2001 and 2002 field seasons. Observations made during transect work and casual observations are included.

Plant species	Plant family	Spring Queen	Worker	Male	New Queen
Agapanthus africanus 'Blue Triumphator'	Liliaceae	*	*		
Anchusia arvensis	Boraginaceae		*		*
Anchusia officinalis	Boraginaceae	*	*		
Anthyllis vulneraria	Fabaceae	*			
Arctium lappa	Asteraceae	*	*		
Ballota nigra	Lamiaceae	*	*	*	*
Betonica officinalis	Lamiaceae		*	*	*
Brassica napus	Brassicaceae		*		
Calystegia silvatica	Convolvulaceae		*	*	
Carduus crispus	Asteraceae			*	
Centaurea nigra	Asteraceae	*	*	*	*
Centaurea scabiosa	Asteraceae	*	*		
Ceratostigma wilmotianum	Plumbaginacea		*	*	*
Cirsium eriophorum	Asteraceae	*			
Daucus carota	Apiaceae		*	*	
Dipsacus fullonum	Dipsacaceae		*	*	
Echinops 'Veich's Blues'	Dipsacaceae	*		*	*
Echinops ritro	Dipsacaceae	*		*	*
Echinum vulgare	Boraginaceae	*	*		
Eryngium agavifolium	Apiaceae	*		*	*
Eryngium alpinum	Apiaceae	*		*	*
Eryngium giganteum	Apiaceae	*		*	*
Galega officinalis	Fabaceae	*	*		
Geranium 'Brookside'	Geraniaceae	*			
Geranium 'Johnson Blue'	Geraniaceae	*			
Geranium karmina	Geraniaceae	*			
Geranium macrorrhizum 'Spessart'	Geraniaceae	*			
Geranium sanguineum 'Vision'	Geraniaceae		*	*	*
Geranium sylvaticum 'Mayflower'	Geraniaceae	*			
Hemerocallis 'Carey Quinn'	Liliaceae				*
Impatiens glandulifera	Balsaminaceae		*	*	
Knautia arvensis	Dipsacaceae		*	*	
Kniophfia 'Alcazar', 'Little Maid', 'Luna', 'Uvaria'	Liliaceae	*			
Lamium album	Lamiaceae	*	*	*	*
Lamium maculatum	Lamiaceae		*	*	*
Lamium purpureum	Lamiaceae	*	*	*	
Lathyrus aphaca	Fabaceae		*		
Lathyrus latifolius	Fabaceae		*		
Lathyrus pratensis	Fabaceae	*	*		
Lavandula x *intermedia* 'Dutch'	Lamiaceae		*	*	*
Lotus corniculatus	Fabaceae	*	*	*	*

Continued on next page

Table 4 continued.

Plant species	Plant family	Spring Queen	Worker	Male	New Queen
Lythrum salicaria	Lythraceae	*	*		
Lythrum virgatum 'Rose Queen'	Lythraceae		*		*
Medicago lupulina	Fabaceae		*		
Medicago sativa	Fabaceae		*		
Melilotus albus	Fabaceae		*		
Nepeta sibirica 'Six Hills Giant'	Lamiaceae	*	*		
Nepeta x faassenii	Lamiaceae	*			
Origanum vulgare	Lamiaceae		*		
Perowskia a. Blue Spire	Lamiaceae		*	*	*
Prunella vulgaris	Lamiaceae		*		
Salivia farinacea	Lamiaceae	*	*		
Salvia lavandulifolia	Lamiaceae	*			
Salvia officinalis 'berggarten'	Lamiaceae	*			
Salvia pratensis (pink)	Lamiaceae	*			
Salvia sclaria 'turkestancia'	Lamiaceae				
Sedum 'spectabile'	Crassulaceae	*		*	*
Solanum dulcamara	Solanaceae		*		
Stachys officinalis	Lamiaceae		*		
Symphytum officinale	Boraginaceae		*		
Trifolium pratense	Fabaceae	*	*	*	*
Trifolium repens	Fabaceae	*	*	*	*
Vicia cracca	Fabaceae	*	*		
Vicia sativa	Fabaceae	*	*	*	
Vicia tetrasperma	Fabaceae		*	*	*

Figure 1. *Bombus humilis* distribution map showing 19th Century records, records for 1900 – 1969 and records for 1970 to present, reproduced from Edwards (2002).

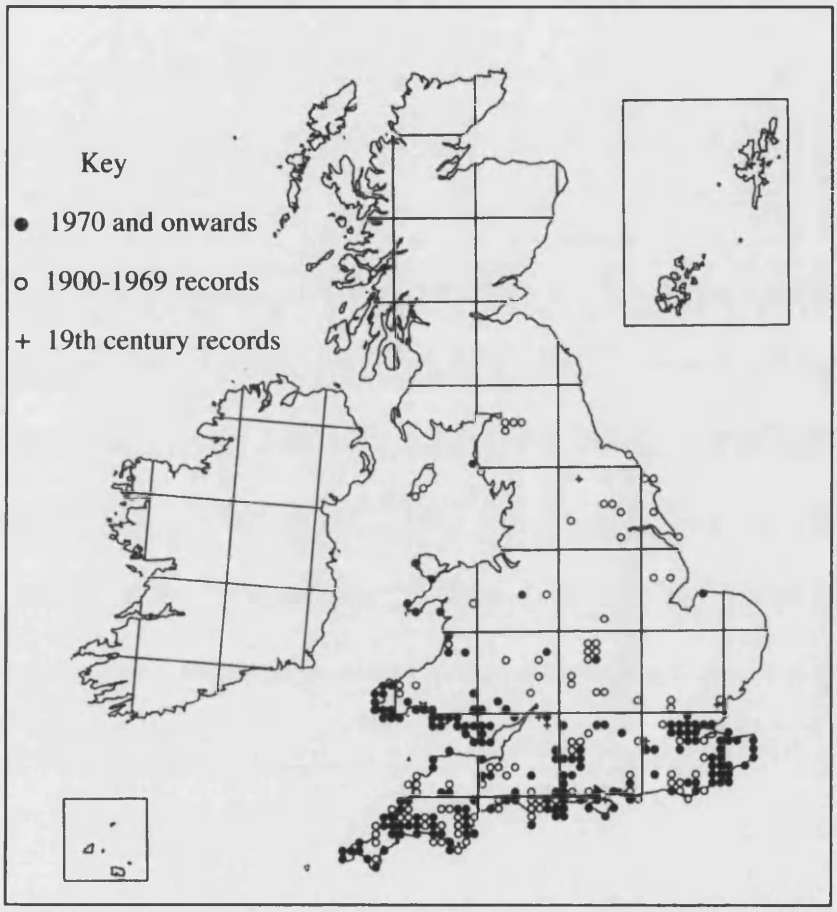


Figure 2a. A map of the Temple Mills site to show the location of the transects.

1 = Stream transect, 2 = Pond transect

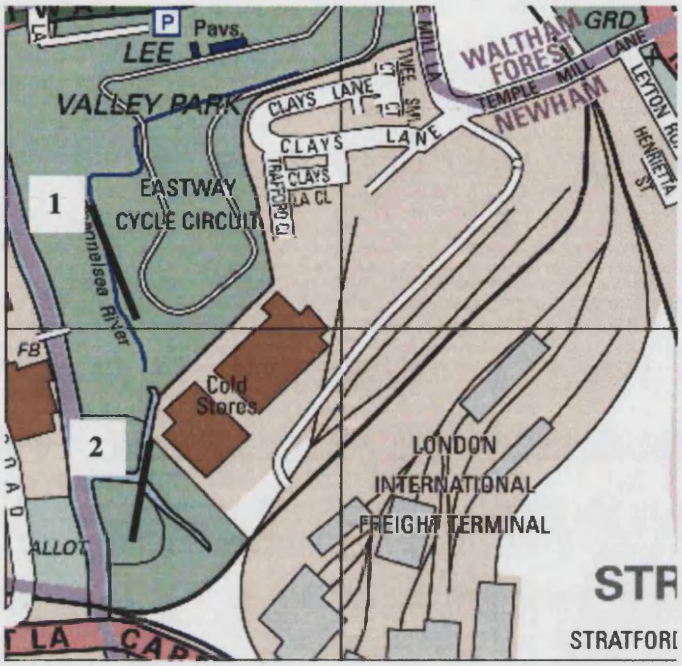


Figure 2b. A map of the Thames Barrier Park site to show the location of transects.

3 = Wildflower transect, 4 = Garden transect.

Figure 3. A map of *Bombus humilis* distribution in London and the Thames Corridor showing records collated by Peter Harvey of the Bees Wasps and Ants Recording Society (1992-1998) and Roselle Chapman (RC) in 2000-2002.

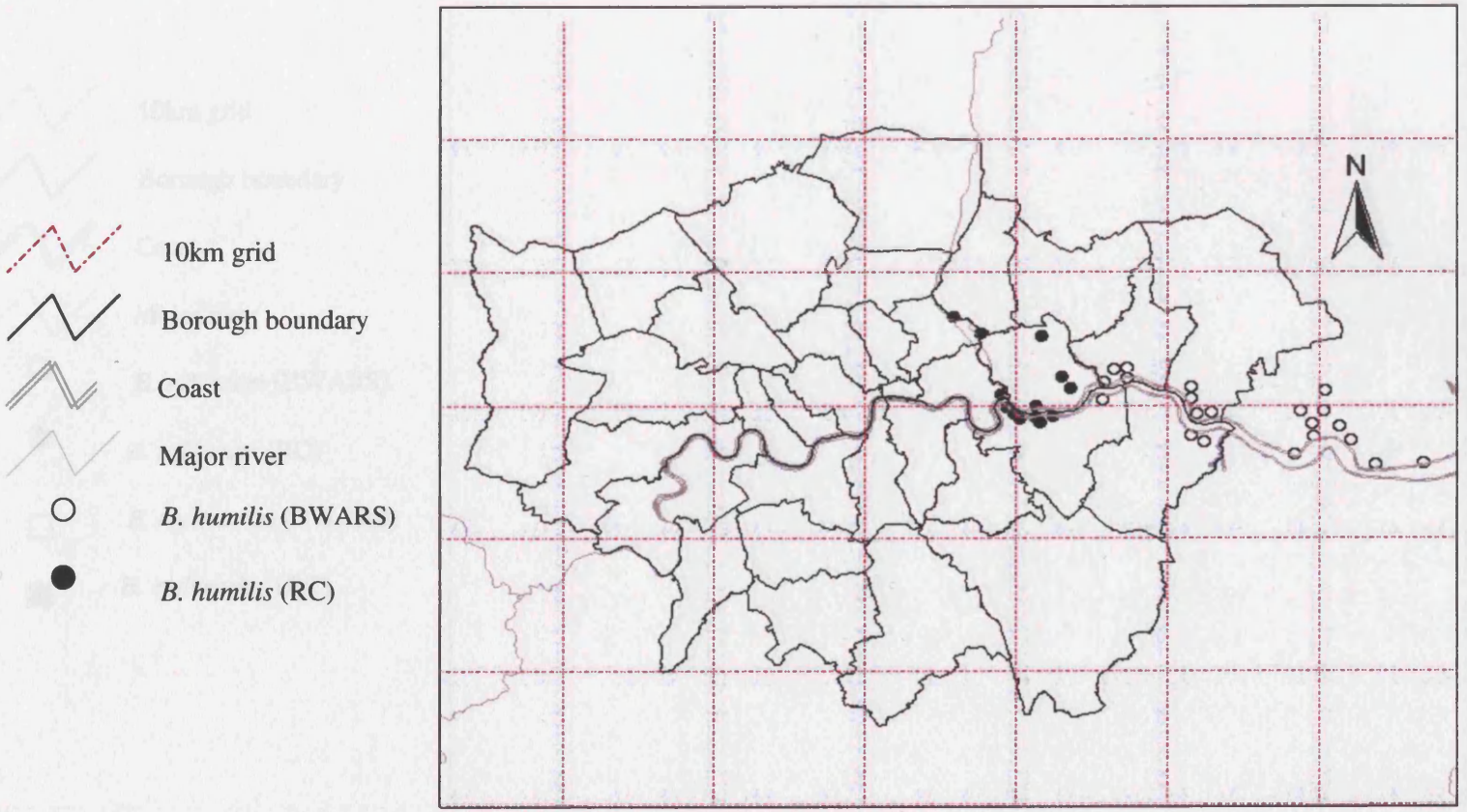


Figure 4. A map of *Bombus sylvarum* and *B. ruderarius* distribution in London and the Thames Corridor showing records collated by Peter Harvey of the Bees Wasps and Ants Recording Society (1992-1998) and Roselle Chapman (RC) in 2001-2002.

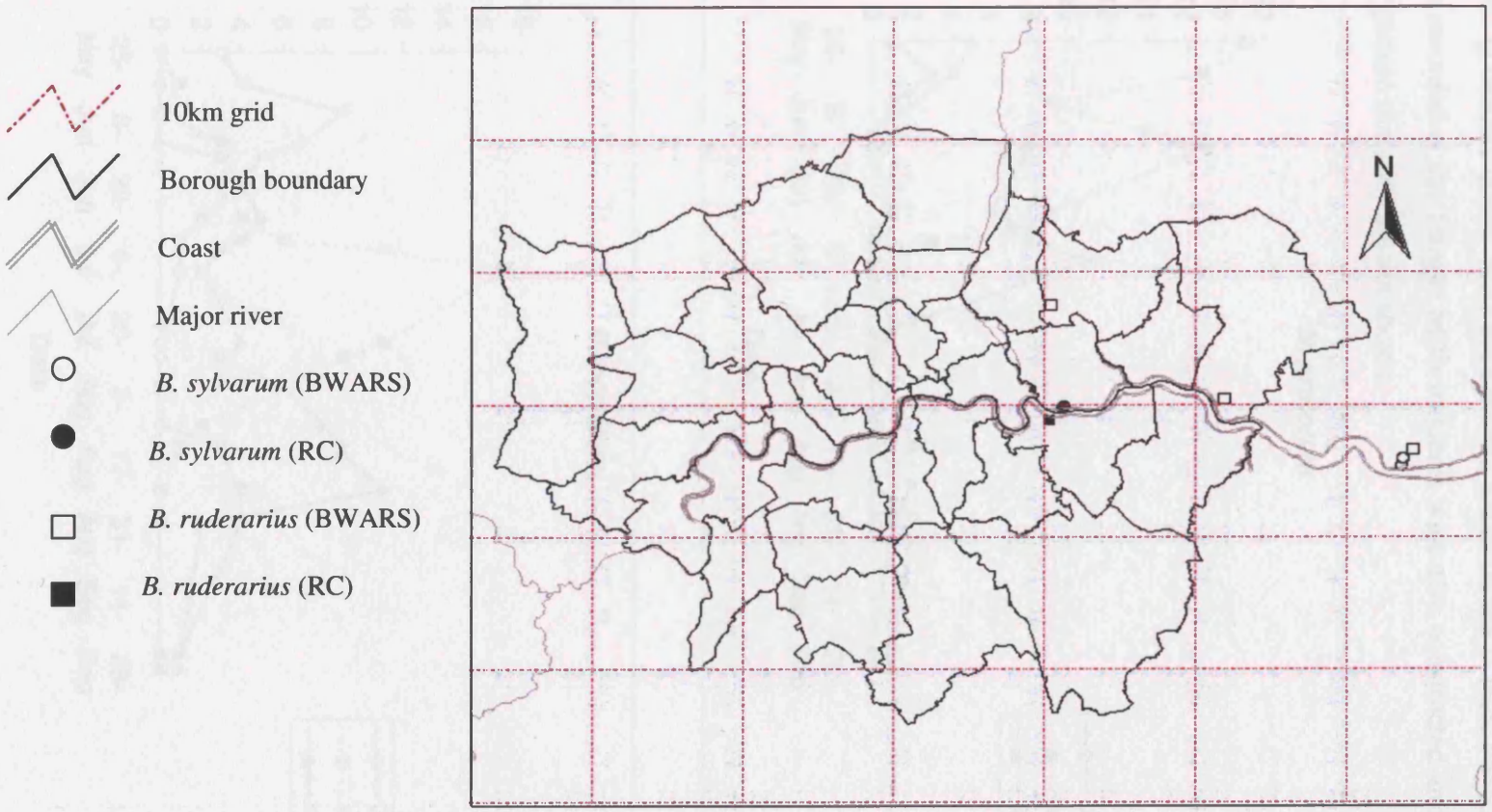
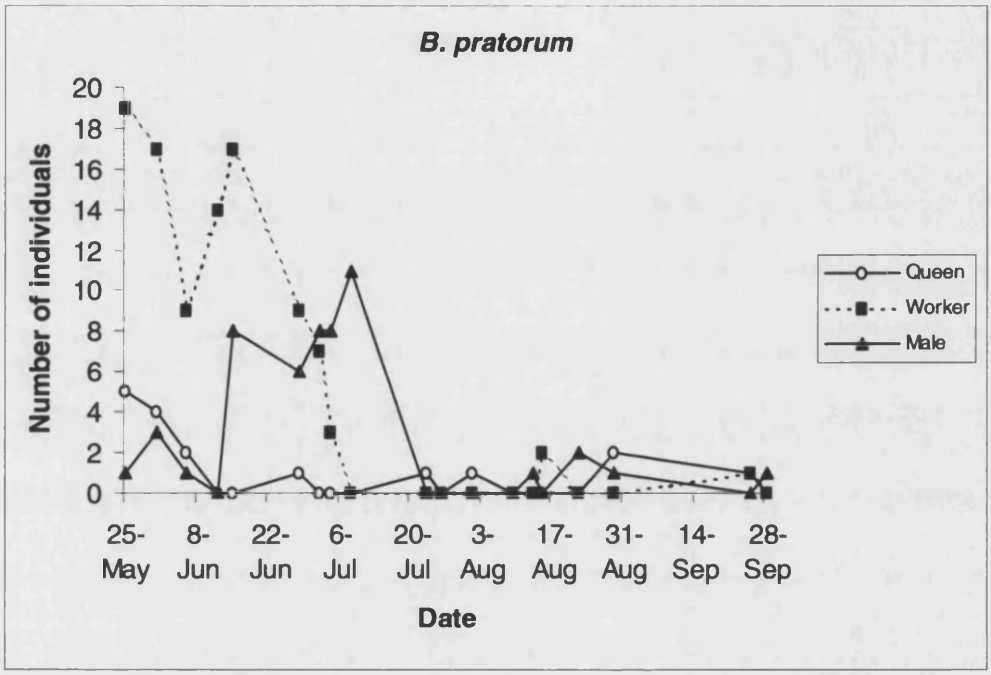
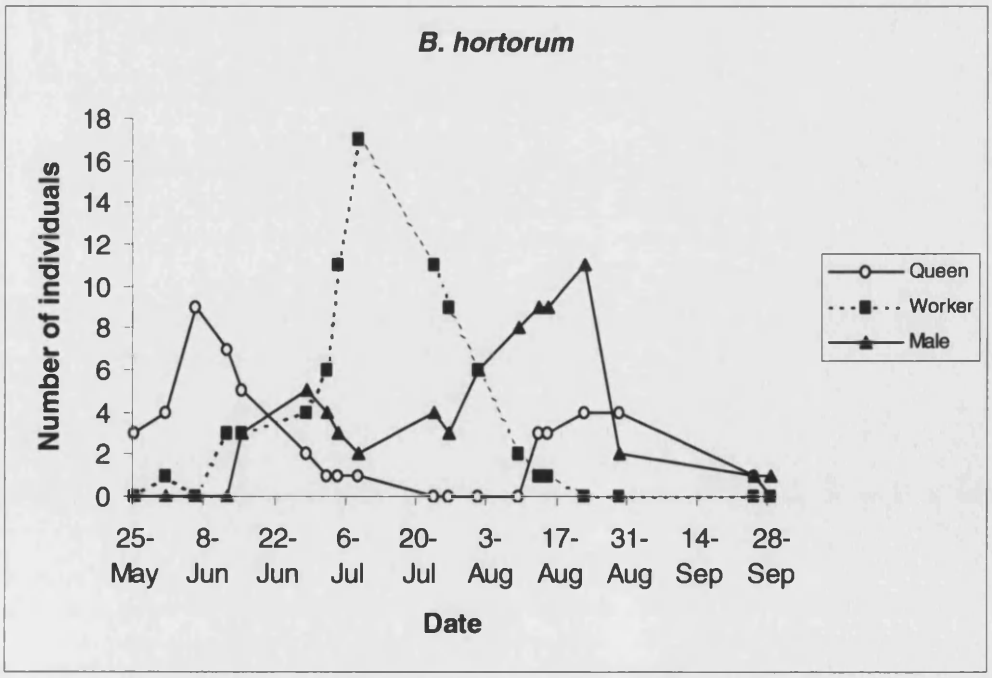


Figure 5. The phenology of each *Bombus* species and caste for the two Thames Barrier Park transects combined. Observations of *B. terrestris* and *B. lucorum* workers were combined for analysis due to their similarities in the field. In the legend *M* denotes male and *Q* denotes queen. Note the different scales on the y-axes. Phenologies for the species recorded at the Temple Mills transects were also constructed and show such a similar pattern that they are not shown.





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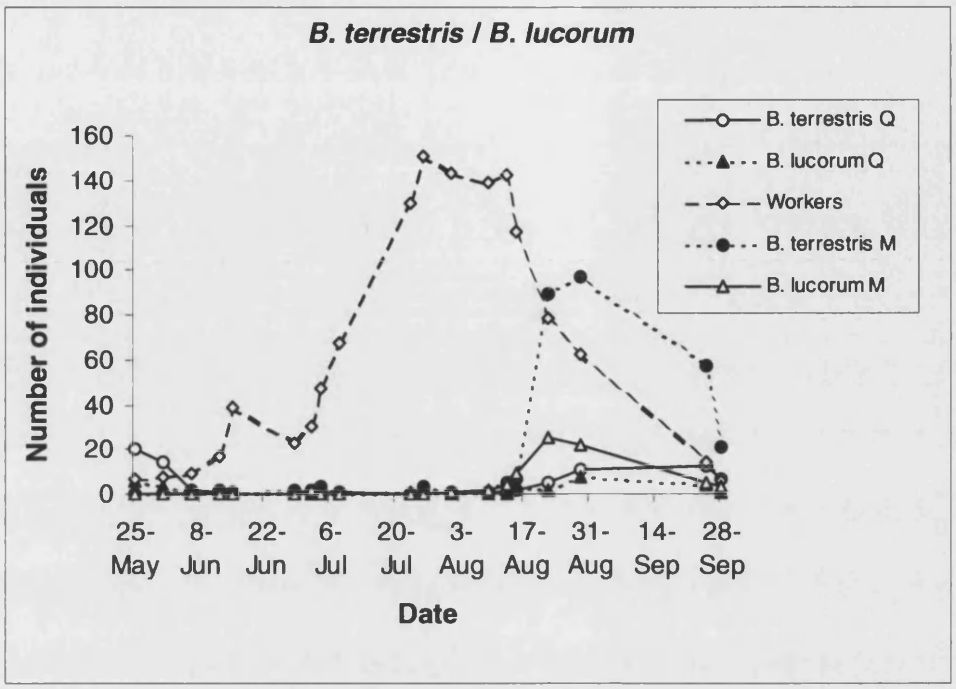

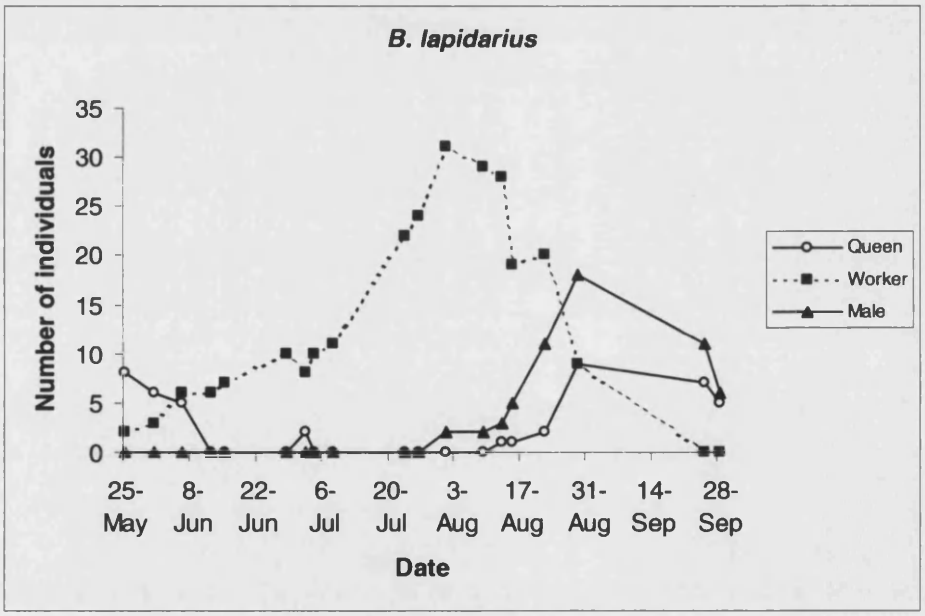

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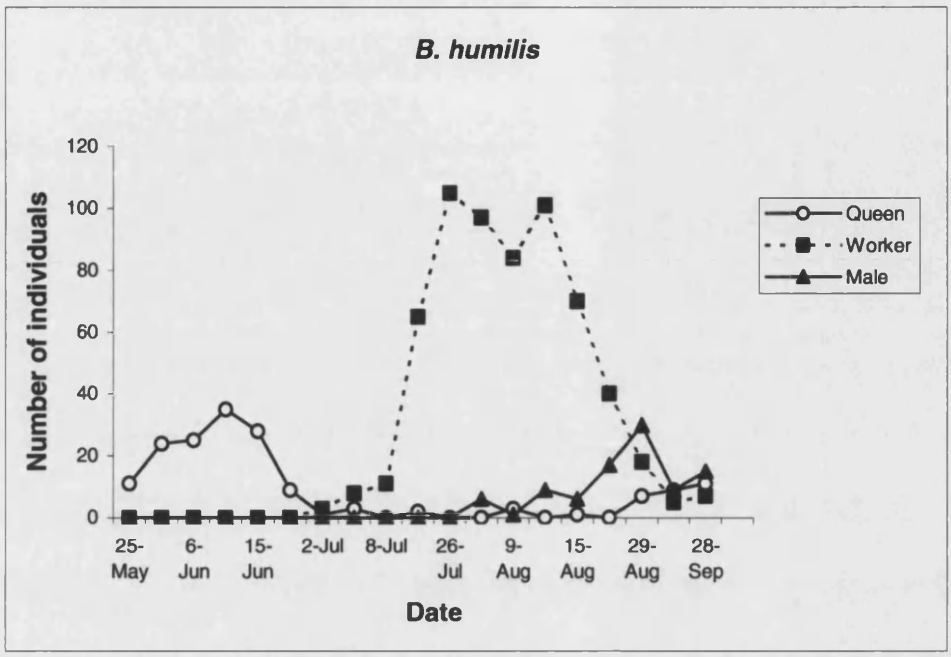


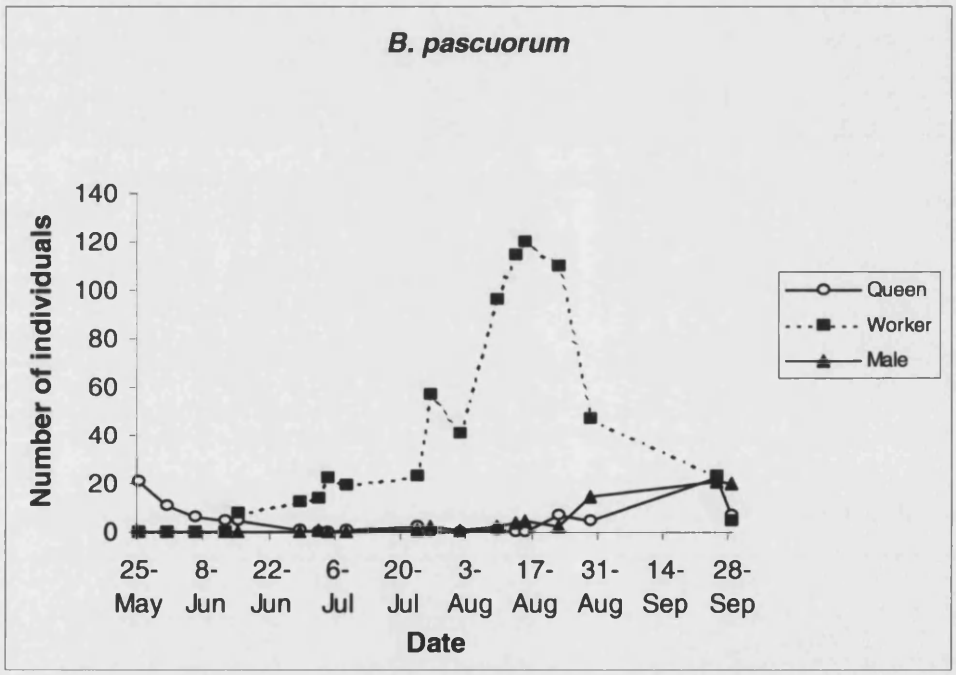


Figure 6. Total *B. humilis* observations by caste at the four study transects. Observations were recorded over nine surveys, conducted between 21st May and 19th August 2001 along four 1m × 100m transects.

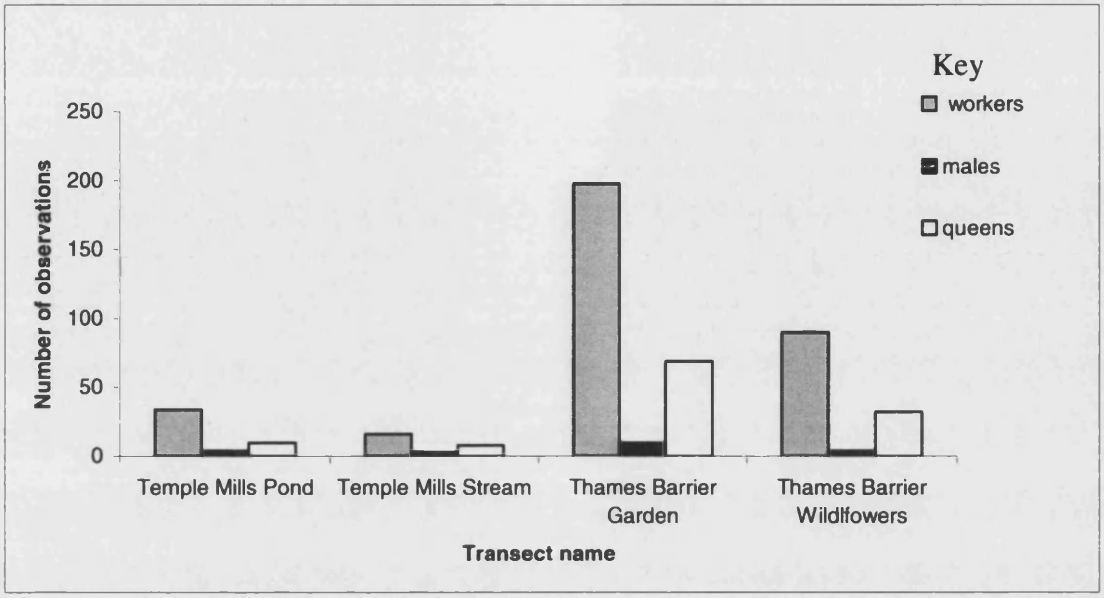


Figure 7. The phenology of flowering plant abundance. Floral abundance is calculated as the number of squares sampled that contained inflorescences. To sample the availability of potential forage I placed a $1m^2$ quadrat at 15m intervals along the 100m transect. The quadrat was sub-divided with string into one hundred $10cm^2$ units, known as squares from here on. Each square was surveyed for the presence or absence of inflorescences. Where inflorescences were present in a square they were identified to species and their presence was mapped by square onto a standardised data sheet. Using this method all of the plants in flower at the time, within the quadrat, were identified to species and the area they covered within the quadrat calculated. Note the different scales on the y-axes

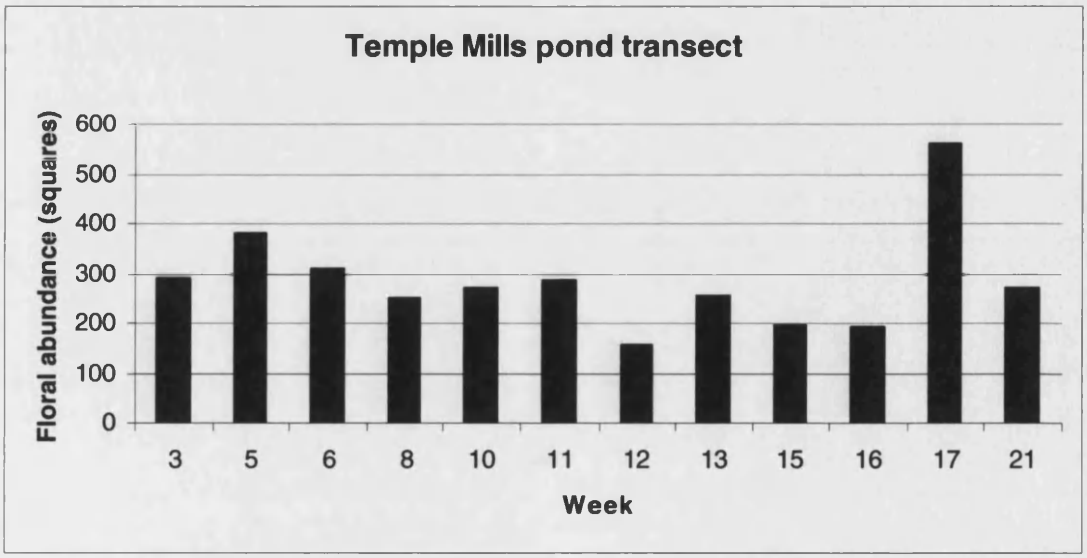
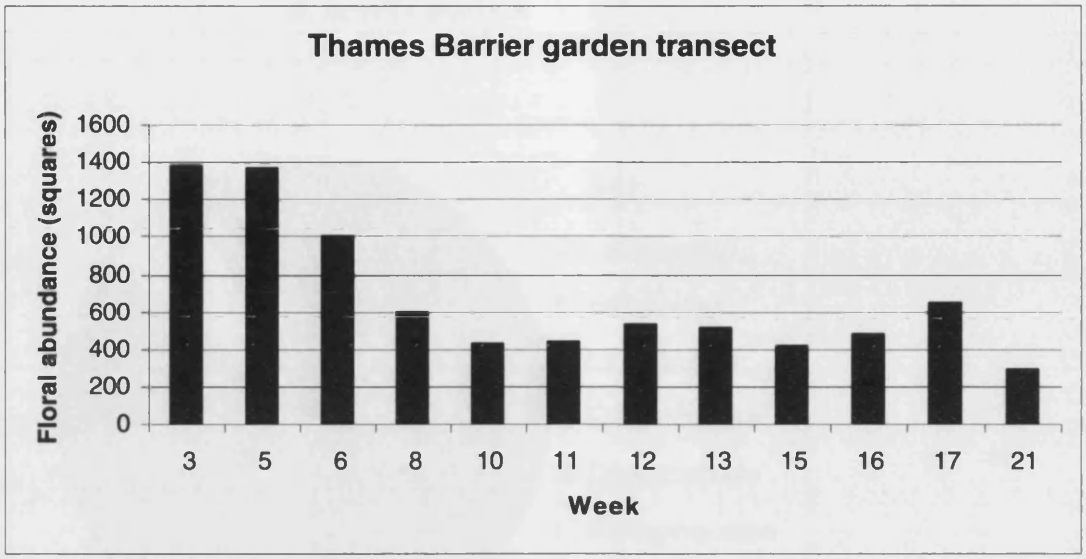


Figure 7 continued



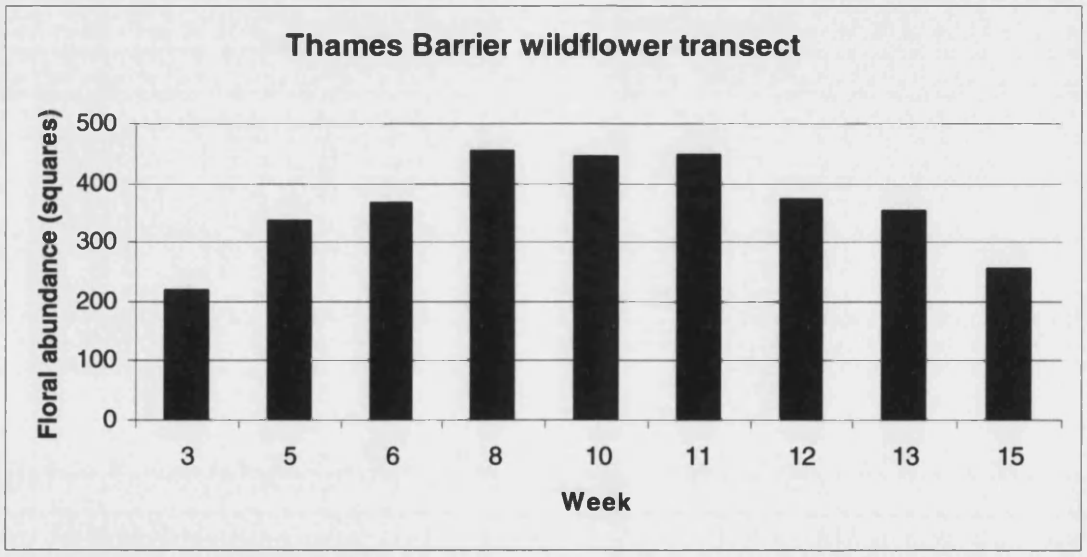


Figure 8. *B. humilis* worker forage observations by plant family.

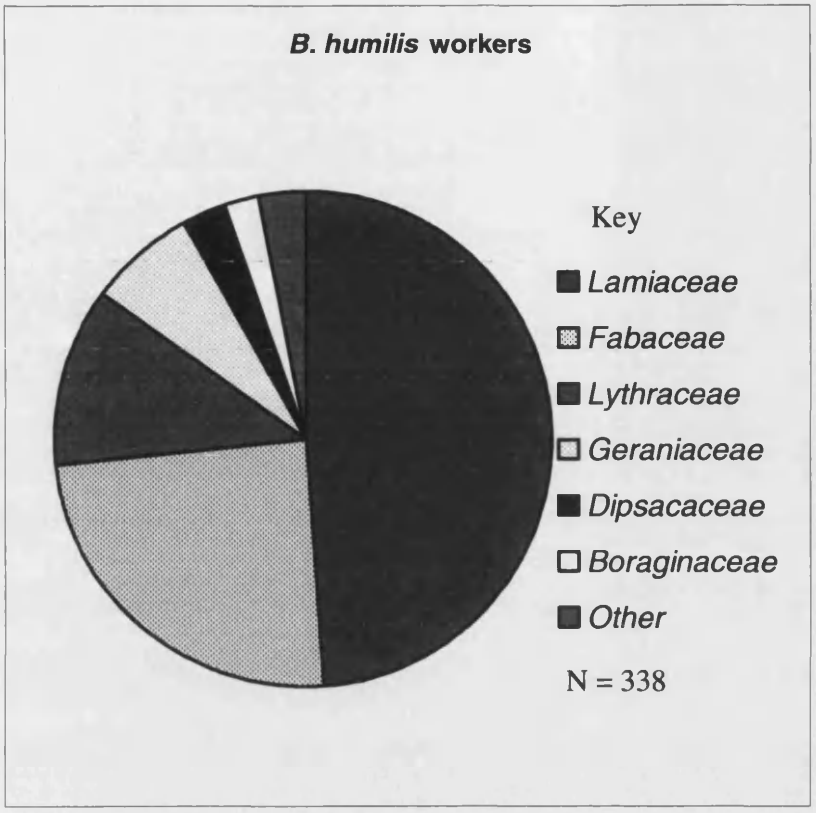
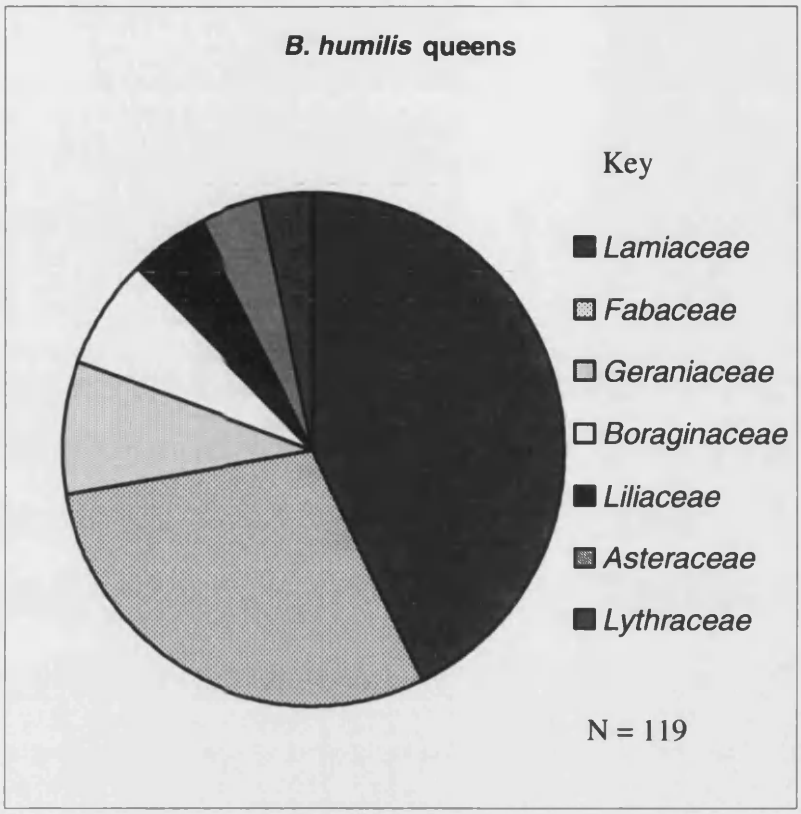


Figure 9. *B. humilis* queen forage observations by plant family.



Chapter 4

A test for interspecific competition between a threatened (*Bombus humilis*) and non-threatened bumble bee species.

SUMMARY

Bumble bees exhibit niche separation by generally feeding on flowers that have corolla lengths corresponding to their proboscis lengths, thereby reducing interspecific competition (Heinrich, 1976b; Inouye, 1978; Ranta, 1984; Ranta & Lundberg, 1980; Teräs, 1976). *B. pascuorum* and *B. humilis* (sub-genus *Thoracobombus*) have medium-length probosces (Medler, 1962) and have a high degree of niche overlap (Goulson & Darvill, 2004). Such are the conservation concerns for *B. humilis* that it has Biodiversity Action Plan Species status (Anonymous, 1999), but *B. pascuorum* remains common throughout Britain (Goulson, 2003). If bumble bees are in competition for floral resources, changes in the foraging behaviour of one of the species should be observed after its potential competitors are removed. This study had two aims and employs a field manipulation experiment to achieve them. The first aim was to establish which species utilize the same floral resources as *B. humilis* and are therefore potentially in competition with it. The second aim was to test experimentally for such competition by removing either *B. humilis*, or other species (predominantly *B. pascuorum*) from patches of flowers. *B. pascuorum* was the most common species observed, followed by *B. humilis.* Using a General Linear Model the removal of *B. humilis* was shown to have no effect on the duration of stay of other species foraging at patches of flowers, whereas the removal of other species significantly increased the time that *B. humilis* workers spent foraging at patches of flowers ($F_{1,19} = 5.99$, $P < 0.05$). However, due to methodological constraints, the results have alternative explanations. The limitations of the experiment are discussed and improvements to the methods suggested.

1. INTRODUCTION

"Competition is an interaction between individuals, brought about by a shared requirement for a resource in limited supply, and leading to a reduction in the survivorship, growth and/or reproduction of at least some of the competing individuals concerned" (Begon *et al.*, 1996). Like other organisms, bumble bees can experience competition between individuals of the same species, known as intraspecific competition, and between individuals of different species, known as interspecific competition. Both intraspecific and interspecific competition can take two broad forms. Firstly, interference (or contest) competition involves the direct interaction between individuals, where one individual will prevent another from exploiting the resource. Aggressive social behaviours are employed, such as dominance hierarchies and territoriality. There are winners and losers. This type of competition is not necessarily linked with resource availability and the presence of an individual may prevent another from exploiting the resource even though there is plenty for both. Secondly, exploitation (or scramble) competition is linked very closely with the level of a resource and occurs only when the resource is in limited supply. Unlike the case when there is interference competition, there is little physical interaction between individuals with regards to resource sharing. Rather the impact on individuals by the presence and activity of others is through the depletion of resources. Those who get to them first gain resources and efficiency in the location and consumption of a resource is important. Foraging bumble bees do not defend territories and are rarely aggressive towards each other and therefore potentially exhibit exploitation but not interference competition.

Bumble bees gain their energy from flowers in the form of nectar, which is extracted with the proboscis (tongue) from the nectary of the flower, and pollen, which is

transferred from the stamen onto their hairy bodies and then scraped into pollen baskets on their hind legs. Body size, head width and proboscis length vary between species of *Bombus* and restrict species to extracting nectar from certain flower types. As a guild bumble bees exploit the same class of environmental resource in the same way. They have been shown to exhibit niche differentiation by generally feeding on flowers that have corolla lengths corresponding to their proboscis lengths, thereby reducing interspecific competition in a community (Heinrich, 1976b; Inouye, 1978; Pyke, 1982; Ranta, 1984; Ranta & Lundberg, 1980; Teräs, 1976). Long-tongued bumble bees can access flowers with deep nectar-containing spurs providing a food source free from competition from short-tongued species and honeybees. Analysis of foraging efficiency has shown that time spent per flower decreases with tongue length (Inouye, 1980a) and that efficiency of nectar extraction drops rapidly with increasing flower depth beyond a bee's proboscis length (Harder, 1983, 1986a). Of the British species, *B. hortorum*, at a mean of 12.3mm, has the longest proboscis (Medler, 1962). The forage plants more frequently visited by this large bumble bee are flowers with deep corollas such as foxgloves and honeysuckle (Chapter 2 of this thesis). Conversely, *B. jonellus* is a smaller bee with the shortest tongue (6.2mm) (Ranta, 1984) and is reported to exploit the shallow flowers of blackberry and bilberry (Alford, 1975). There is, of course, flexibility in this system, and when deep flowers are unavailable longer-tongued bees are able to exploit shallow flowers, although rather awkwardly and less efficiently than the shorter tongued species (Ranta & Lundberg, 1980). Similarly, short-tongued bumble bees that specialise in collecting nectar from shallow open flowers have the ability to bite into the corolla tube of deeper flowers and extract the nectar that would otherwise be inaccessible to them via the normal route. This is known as 'nectar robbing' (Inouye, 1980b).

B. pascuorum and *B. humilis* are two species of the sub-genus *Thoracobombus* that are physically and ecologically very similar. As carder bees, both species have similar nesting requirements (Prys-Jones & Corbet, 1991). Both species have medium-length probosces (of 8.54mm and 8.17mm, respectively) (Medler, 1962) and have been shown to utilise species of the Lamiaceae and Fabaceae families more frequently than others (Chapters 2 and 3 of this thesis). In a study on Salisbury Plain, Goulson and Darvill (2004) showed that, when collecting both pollen and nectar the two species have one of the highest levels of niche overlap of all species recorded. Despite these close similarities, *B. humilis* has undergone a recent decline in numbers and a contraction of range (Edwards, 2002b), whereas *B. pascuorum* remains common throughout Britain (Edwards, 2003). This raises the question of why the decline of some species has been much more severe than others. Williams (1986) applied a 'marginal mosaic model' to bumble bees that assumed that the foraging profits of a species would be reduced at the edge of its range where conditions are sub-optimal, and that consequently local abundances of that species would also be reduced by any degradation of habitat. Populations of *B. humilis* in southern England and Wales are at the edge of the geographical range of the species, which extends north only into southern Scandinavia (Williams, 1986), and these populations seem to survive only in the best habitats of unimproved chalk grassland (Edwards, 2002b) and forage rich areas of the Thames Corridor (Chapter 3, this thesis). The general consensus is, therefore, that loss of habitat exacerbated by the fact that *B. humilis* is on the edge of its geographic distribution has resulted in the reduction in its abundance and range. The reduction in local abundance makes a species more vulnerable to perturbations such as loss of habitat, parasites and competition. It may even be the case that at a site one species, which is at the centre of

its range and therefore better suited to conditions, is able to out compete another species at the edge of its range (Goulson, 2003). The range of *B. pascuorum* includes all of Great Britain and extends further north, deep into Scandinavia, and therefore, according to the 'marginal mosaic model', this species should not be so sensitive to habitat degradation in Great Britain. *B. humilis*, *B. pascuorum* and seven other species of *Bombus* have been identified in Greater London (Chapters 2 and 3 of this thesis) and *B. humilis* and *B. pascuorum* have been shown to utilise a similar range of forage plants (Chapter 3, this thesis). If these bumble bees are in competition for floral resources, changes in the foraging behaviour of a species should be observed after its potential competitors are removed. Employing a field manipulation experiment, this study had two aims. First, to establish which species utilize the same floral resources as *B. humilis* and are therefore potentially in competition with it. Second, to test experimentally for such competition: I removed all of the potential competitors of *B. humilis*, including *B. pascuorum* from flower patches, and predicted that, if interspecific competition were occurring, the duration of foraging visits of *B. humilis* should increase. To test if competition were occurring symmetrically, I then performed the reciprocal experiment, removing *B. humilis* from flower patches visited by other species.

2. MATERIALS AND METHODS

2.1 Selection of study site

The experiment was carried out in landscaped gardens at the Millennium Village in east London (SE10) in August 2001 and July and August 2002. The main feature of the gardens was a fine-turf lawn surrounded by a border composed of flowering shrubs and mixed wildflowers. The Millennium Village was selected as the study site for three main reasons. First, it was accessible. Second, eight species of *Bombus* (*B. terrestris, B. lucorum, B. pascuorum, B. humilis, B. lapidarius, B. pratorum, B. hortorum, B. ruderarius*) were recorded and *B. humilis* was known to be abundant (Chapter 3 of this thesis). Third, large but discrete patches of low-level, clump-forming plants favoured by *B. humilis* were abundant, for example *Lotus corniculatus, Trifolium pratense,* and *T. repens*. The timing of the experiment was set to coincide with the seasonal peak in *B. humilis* worker numbers (Chapter 3 of this thesis).

2.2 Experimental procedure

Two people were required to carry out the work effectively and accurately. A patch of flowers composed of *Lotus corniculatus, Trifolium pratense,* or *T. repens* and measuring approximately $2m^2$ was identified. The first part of the experiment was carried out in 2001 and involved applying two experimental treatments, A and B, to the patch of flowers. Treatment A was the control treatment: over a period of 30 min, every worker bee that entered the patch was identified to species and timed for its duration of stay in the patch. No interference was made. During treatment B, two people observed the patch for 30 min. Each worker that arrived at the patch was identified to species. The arrival time of each *B. humilis* worker was noted by person 1, who carefully observed it until its departure, when the time was noted again. Simultaneously, person

2 removed all species, except *B. humilis*, the instant that they alighted on the patch (Figure 1).

In 2002 the experiment described above was repeated but with an additional treatment (reciprocal removal of *B. humilis*). Three experimental treatments, A, B and C, were applied to the patch, where treatments A and B were the same as above. In treatment C two people observed the patch for 30 min. Each worker that arrived at the patch was identified to species. Person 1 removed all *B. humilis* workers the instant that they alighted on the patch. Simultaneously, the arrival time of workers of all other species was noted by person 2, who carefully observed each individual until its departure, when the time was noted again (Figure 1). In both years, each removed worker bee was removed with, and stored in, a separate clear plastic (Universal) tube with holes drilled in the top. This procedure did not interfere with other workers in the vicinity. The tubes were kept in a cool dark container until the period of 30 min was completed, after which workers were released. In both years, the experiment was replicated ten times, i.e. using ten different $2m^2$ patches of flowers. The order in which the treatments A, B, and C were applied was alternated over the ten patches to eliminate any accumulative effects of disturbance caused by the experimenters' presence and actions. All manipulations were carried out between 08:50 and 17:50.

2.2 Statistical methods

Workers that were already present at the beginning of the 30 min period or still present at the end of the 30 min period were included in the analysis for the time that they were recorded at the patch. The data (duration of visits by worker bees) were analysed using a General Linear Model (GLM) designed as a mixed model two-way ANOVA. The

fixed factor was the experimental treatment ('treatment', i.e. removal or non-removal of worker bees) and the random factor was the flower patch ('patch'). The *F* statistic for the treatment term was therefore calculated as the mean square of the treatment term divided by the mean square of the interaction term (Sokal & Rohlf, 1981). The data were analysed for normality using the Kologorov-Smirnov normality test. Of the forty datasets that could be analysed, thirty-three were normal and therefore the data were not transformed.

3. RESULTS

A total of 762 bumble bee workers from six species were recorded during this study. The most common species observed was *B. pascuorum*, which made up 54.6% of all observations, followed by *B. humilis* (30.7%). *B. pascuorum* was therefore the chief potential competitor of *B. humilis* at the study site. Three other species made up less than 15% of all observations between them, i.e. *B. terrestris / B. lucorum* (9.8%), *B. lapidarius* (1.9%), *B. hortorum* (3%) (Figure 2). No acts of aggression between any species were observed but it was noted that *B. humilis* seemed more easily scared off by people and bees than other species.

3.1 The effect of the removal of potential competitors of *B. humilis*

In 2001 across all the 30 min observation periods, the mean time that *B. humilis* spent foraging in the presence of competitors was 158.5 seconds (SD 57.1, N = 10) and in the absence of competitors, this increased to 222.0 seconds (SD 109.5, N = 10). In 8 out 10 patches the duration of stay was longer in the manipulated patches than in the unmanipulated patches (Figure 3a). In 2002, across all 30 min observation periods, the mean time that *B. humilis* spent foraging in the presence of competitors was 218 seconds (SD 132.6, N = 10); in the absence of competitors this increased to 334 seconds (SD 112.0, N = 10). In 9 out 10 patches the duration of stay was longer in the manipulated patches than in the unmanipulated patches (Figure 3b). In the 2001 experiment, the treatment (removal of potential competitors) had no significant effect on the duration of visits by *B. humilis* workers ($F_{1,9} = 2.25$, $P > 0.05$) (Table 1a). The effect of 'patch' was not significant but the interaction of 'patch' and 'treatment' was significant although this result appears to have little scientific significance since neither 'patch' nor 'treatment' had significant effects (Table 1a). In 2002, the 'treatment' also

had no significant effect on the duration of visits by *B. humilis* workers (comparison of treatments A and B: ($F_{1,9} = 3.61$, $P > 0.05$)) (Table 1b). The effect of 'patch' and the interaction between 'patch' and 'treatment' were not significant (Table 1b). Given that the visit durations of *B. humilis* workers were increased by the removal of potential competitors in 9 patches out of 10 in 2001 and 9 out of 10 patches in 2002 (Figures 3a, 3b), the data were pooled across years to increase the power of the test. In the pooled data, there was a significant effect of the treatment ($F_{1,19} = 5.99$, $P < 0.05$) (Table 1c); therefore the removal of potential competitors significantly increased the time that *B. humilis* workers spent foraging at patches of flowers. In the pooled 2001 and 2002 data, the effect of 'patch' was also significant but the interaction of 'patch' and 'treatment' was not (Table 1c). The significant effect of 'patch' indicates that some patches consistently attracted longer or shorter forage visits than other patches. This was probably due to non-uniformity of the flower patches. For example, the patches were composed of different numbers of flowers, which had started flowering at different times and therefore offered different quantities of reward.

3.2 The effect of the removal of *B. humilis* workers

The effect of removing *B. humilis* workers can only be gauged from the 2002 data. The treatment had no significant effect (comparison of treatments A and C: $F_{1,9} = 0.18$, $P > 0.05$) (Table 1d) and therefore the removal of *B. humilis* had no effect on the duration of stay of other species foraging at patches of flowers (Figure 3c). Again, the effect of 'patch' was significant (Table 1d), which was likely to have occurred for the reasons described above. There was no obvious trend in the data, with 'other species' foraging for longer in the absence of *B. humilis* on four out of ten occasions.

In six out of ten patches there was no significant difference between the number of *B. humilis* workers and the number of workers of 'other species' removed in treatments C and B respectively (Patch 1: $\chi^2 = 6.0$, d.f. = 1, $P < 0.05$; Patch 2: $\chi^2 = 3.6$, d.f. = 1, $P <$ 0.05 Patch 3: $\chi^2 = 0.82$, d.f. = 1, N.S; Patch 4: $\chi^2 = 0.4$, d.f. = 1, N.S; Patch 5: $\chi^2 =$ 3.57, d.f. = 1, N.S; Patch 6: $\chi^2 = 3.0$, d.f. = 1, N.S; Patch 7: $\chi^2 = 0.67$, d.f. = 1, N.S; Patch 8: $\chi^2 = 3.77$, d.f. = 1, N.S; Patch 9: $\chi^2 = 7.14$, d.f. = 1, $P < 0.05$ Patch 10: $\chi^2 =$ 3.6, d.f. = 1, $P < 0.05$) (Table 2b). However, over all of the patches combined, there were significantly fewer *B. humilis* workers removed than workers of 'other species', with a mean number of 2.3 *B. humilis* workers removed per patch and a mean number of 7.9 workers of 'other species' removed per patch ($\chi^2 = 30.0$ d.f. = 9, $P < 0.001$). There was no significant difference between the number of *B. humilis* workers under control (A) and treatment (B) conditions at each patch, and across all patches combined ($\chi^2 = 0.3$ d.f. = 1, >0.05) (Table 2a). There was also no significant difference between the number of workers of 'other species' under control (A) and treatment (C) conditions at each patch, and across all patches combined ($\chi^2 = 1.3$ d.f. = 1, >0.05) (Table 2b.)

4. DISCUSSION

B. pascuorum was the most abundant species observed during the experiments and is the greatest potential competitor of *B. humilis* for forage. From the pooled data (2001 and 2002) the presence of individuals of other species, primarily *B. pascuorum*, has been shown to curtail the length of time that *B. humilis* can spend foraging at one patch. The reverse is not the case and the removal of *B. humilis* from a patch of forage does not increase the foraging time of other species. However, the imbalance in the number of bees removed in treatments B and C makes the interpretation of the data difficult. It could be argued that by removing competitors of *B. humilis*, forager density is reduced within the patch leaving the standing crop of nectar undepleted, thereby promoting longer visits among the remaining workers (*B. humilis*). In 2002 there was no significant difference between the number of *B. humilis* workers and the number of workers of 'other species' removed in treatments C and B respectively in six out of ten patches. However, over all of the patches combined there were significantly fewer *B. humilis* workers removed than workers of 'other species' (Figure 2b). This comparison cannot be made for the 2001 data because treatment C was not applied. There is also a very slight but not significant positive relationship between the number of bumble bees removed in the treatments and the duration of visit by *B. humilis* in 2001 ($r = 0.08$, d.f. $= 8$, $P > 0.05$) and 2002 ($r = 0.58$, d.f. $= 8$, $P > 0.05$) (Figures 4a and 4b respectively). Due to the fact that significantly fewer *B. humilis* were removed than 'other species', it could therefore be that the removal of *B. humilis* had no significant effect on the duration of foraging by other species because it is present in densities too low to make a difference.

It is possible that *B. humilis* might be competitively repressed by the presence of other species but that it does not pose significant competition to other species, although the present study does not conclusively confirm this. If this is the case and *B. pascuorum* is its main competitor, then this is not just a straightforward example of competitive exclusion due to differing tongue lengths as has been shown to occur (Inouye, 1978) because *B. humilis* and *B. pascuorum* have very similar tongue lengths. There may be an interspecific dominance-subordinance relationship caused by more subtle interactions than aggression. Inouye (1978) demonstrated that the way bumble bees exploit their resources is affected by the presence of other species. He observed two species of differing tongue lengths and showed that bumble bees visited more flowers per stay in the patch in the absence of competitors. Bowers (1985) conducted a removal experiment across a larger spatial (several meadows) and temporal scale than the present study. The two study species, *B. flavifrons* and *B. rufocinctus*, were identical in tongue length, wing length and body size and were not aggressive toward each other. He observed that in the absence of *B. flavifrons, B. rufocinctus* foraged on the same species of flower as *B. flavifrons.* However, in the presence of *B. flavifrons, B. rufocinctus* was observed to forage on less profitable flower species. Interestingly, he also identified a reduced body size of *B. rufocinctus* workers in competition with *B. flavifrons,* which he attributed to a lack of resources. He proposed that this competitive dominance by *B. flavifrons* was related to the differences in phenology of the two species. The dominant species, *B. flavifrons*, initiated colonies earlier than *B. rufocinctus* and maintained higher densities throughout the flying season, thereby maintaining the competitive advantage. Similarly, in the present study the workers of *B. pascuorum* start to emerge a whole month earlier than those of *B. humilis* although the peak in worker numbers of both species occurs at about the same time (mid-August)

(Chapter 3, this thesis). However, even at their peak *B. humilis* are less abundant than *B. pascuorum* (Chapter 3, this thesis). In the present case several species, but predominantly *B. pascuorum*, which are at the centre of their range and therefore better suited to conditions, are possibly out competing another species (*B. humilis*) at the edge of its range, as suggested by Goulson (2003). This may be a reason why *B. humilis* is only observed at the very best flower-rich habitats (Chapter 3, this thesis) so that if it is relegated to less profitable flowers by *B. pascuorum* and others there are still enough resources for all. Further work needs to be carried out, with more rigorous experiments to clarify the exact nature of the relationship of *B. humilis* and its competitors during foraging. This will improve our understanding of why *B. humilis* occurs at some sites and not at other apparently suitable ones.

4.1 Improvements to the experimental procedure

As discussed above further work needs to be conducted to clarify the results. Experimental methods can almost always be improved and the present study is no exception. Therefore, if this experiment were to be repeated some ways by which the method could be improved are outlined below.

One of the main problems posed by the present study is the asymmetry that arises from the pooling of two datasets (treatments A & B 2001, 2002) for comparison with one dataset (A & C 2002). It is possible that this lead to a significant conclusion when considering the impact of the removal of 'other species' on *B. humilis* but not when considering the impact of the removal of *B. humilis* on the duration of stay of 'other species'. Ideally the experiment should be repeated and treatments A, B and C should

be applied to each patch equally. Furthermore, for every manipulation there should be a control to allow for pair wise comparisons.

It has been discussed that over all of the patches combined there were significantly fewer *B. humilis* workers removed than workers of 'other species' in treatments C and B respectively (Results 3.2) (Figure 2b). This imbalance could have lead to *B. humilis* being observed to stay longer at a patch. By removing more individuals in treatment B there are fewer bumble bees to consume the nectar, which could lead to *B. humilis* staying for longer at the patch. To distinguish whether this observation is just a function of the number of individuals present or a more subtle interaction between species, the number of bumble bees needs to be controlled for. Similarly, it is assumed that in the present study *B. pascuorum* is the greatest potential competitor of *B. humilis* because it is the most abundant species. To confirm this the species of bumble bees being compared should be controlled so that, for example *B. humilis* and *B. pascuorum* are compared, then *B. humilis* and *B. lapidarius* etc. The control of the number and species of bumble bees entering a patch could be carried out by either conducting the experiment in an enclosed environment or by removing excess individuals.

Another way in which the present experiment could be improved is through the quantification of resources available in the patch and the quantification of resources utilised by the foragers. In the present analysis the significant effect of 'patch' indicates that some patches consistently attracted longer or shorter forage visits than other patches. This was attributed to the non-uniformity of the flower patches. This needs to be improved upon in order to fully understand the interactions observed. In a study to examine spatial and temporal components of resource assessment by flower-feeding

insects, Thomson *et al.* (1987) counted the number of open inflorescences in their study arena and employed nectar sampling. They acknowledged that their analysis assumed the area was homogenous, whereas nectar and pollen availability were observed to be very patchy (Thomson *et al.*, 1987). The present experiment could be improved by using patches composed of arrays of artificial flowers, uniformly distributed across a defined study area. This would allow for the quantification of resources. Not only would the exact number of inflorescences be known, but also the quality and quantity of nectar available could be controlled. Furthermore, the quantity of nectar consumed could also be measured allowing for any differences in consumption by different species to be recorded.

Finally, the present study just records the duration of stay of an individual within a patch of forage. The recording of each bumble bee's behaviour during its visit to the patch would help to quantify foraging success in the presence and absence of other individuals and species. The time spent searching, probing and feeding should be recorded.

Table 1a. GLM results table for *B. humilis* foraging duration in the presence and absence of competitors, 2001. The fixed factor was the experimental treatment ('treatment') and the random factor was the flower patch ('patch'). The *F* statistic for the treatment term was calculated as the mean square of the treatment term divided by the mean square of the interaction term (Sokal & Rohlf, 1981).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Patch (random factor)	9	459099	422805	46978	1.89	0.057
Treatment (fixed factor)	1	147356	122092	122092	2.25	>0.05
Patch*Treatment	9	487607	487067	54179	2.18	0.026
Error	165	4101755	4101755	24859		
Total	184	5195816				

Table 1b. GLM results table for *B. humilis* foraging duration in the presence and absence of competitors, 2002 (comparison of treatments A and B). The F value for the treatment term was calculated as described in the legend of Table 1a.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Patch (random factor)	9	804081	759773	84419	1.62	0.126
Treatment (fixed factor)	1	226462	185617	185617	3.61	>0.05
Patch*Treatment	9	462926	462926	51436	0.99	0.459
Error	75	3913814	3913814	52184		
Total	94	5407283				

Table 1c. GLM results table for *B. humilis* foraging duration with and without competitors, 2001 and 2002 combined. The F value for the treatment term was calculated as described in the legend of Table 1a.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Patch (random factor)	19	1745180	1638278	86225	2.58	0.000
Treatment (fixed factor)	1	347812	307708	307708	5.99	0.003
Patch*Treatment	19	976538	976538	51397	1.54	0.073
Error	240	8015569	8015569	33398		
Total	279	11085100				

Table 1d. GLM results table for 'other species' foraging duration in the presence and absence of *B. humilis*, 2002 (comparison of treatments A and C). The F value for the treatment term was calculated as described in the legend of Table 1a.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Patch (random factor)	9	3198969	3327726	369747	2.71	0.006
Treatment (fixed factor)	1	392796	33100	33100	0.177	>0.05
Patch*Treatment	9	1684618	1684618	187180	1.37	0.205
Error	154	20987565	20987565	136283		
Total	173	26263947				

Table 2a. The number of bumble bees observed at each patch and the number removed (2001). 'Others' denotes bumble bees of all species except *B. humilis*.

	Treatment A unmanipulated (no bees removed)			Treatment B manipulated (others removed)	
	n bees present *B. humilis*	n bees present others	n bees present all bees	n bees present *B. humilis*	n bees removed others
Patch					
1	15	4	19	11	9
2	9	7	16	14	10
3	13	10	23	6	9
4	12	13	25	7	7
5	11	9	20	9	15
6	9	4	13	3	11
7	1	11	12	5	13
8	10	12	22	14	8
9	12	6	18	10	6
10	6	5	11	7	6
Total	98	81	179	86	94

Table 2b. The number of bumble bees observed at each patch and the number removed (2002). 'Others' denotes bumble bees of all species except *B. humilis*.

	Treatment A unmanipulated (no bees removed)			Treatment B manipulated (others removed)		Treatment C manipulated (*B. humilis* removed)	
	n bees present *B. humilis*	n bees present others	n bees present all bees	n bees present *B. humilis*	n bees removed others	n bees present Others	n bees removed *B. humilis*
Patch							
1	5	11	16	5	6	19	0
2	6	10	16	3	8	4	2
3	2	5	7	5	7	6	4
4	8	6	14	7	6	12	4
5	5	6	11	3	6	14	1
6	2	12	14	3	9	15	3
7	6	11	17	6	4	12	2
8	0	7	7	6	10	3	3
9	0	7	7	5	12	8	2
10	0	4	4	5	8	4	2
Total	34	79	113	48	76	97	23

Figure 1. A diagram to illustrate the treatments applied to each patch of forage. Each treatment was applied for 30 minutes. Treatment A was the control where every bee that entered the patch was identified to species and timed for its duration of stay in the patch. During treatment B all species, except *B. humilis*, were removed the instant that they alighted on the patch. In treatment C, *B. humilis* was removed.

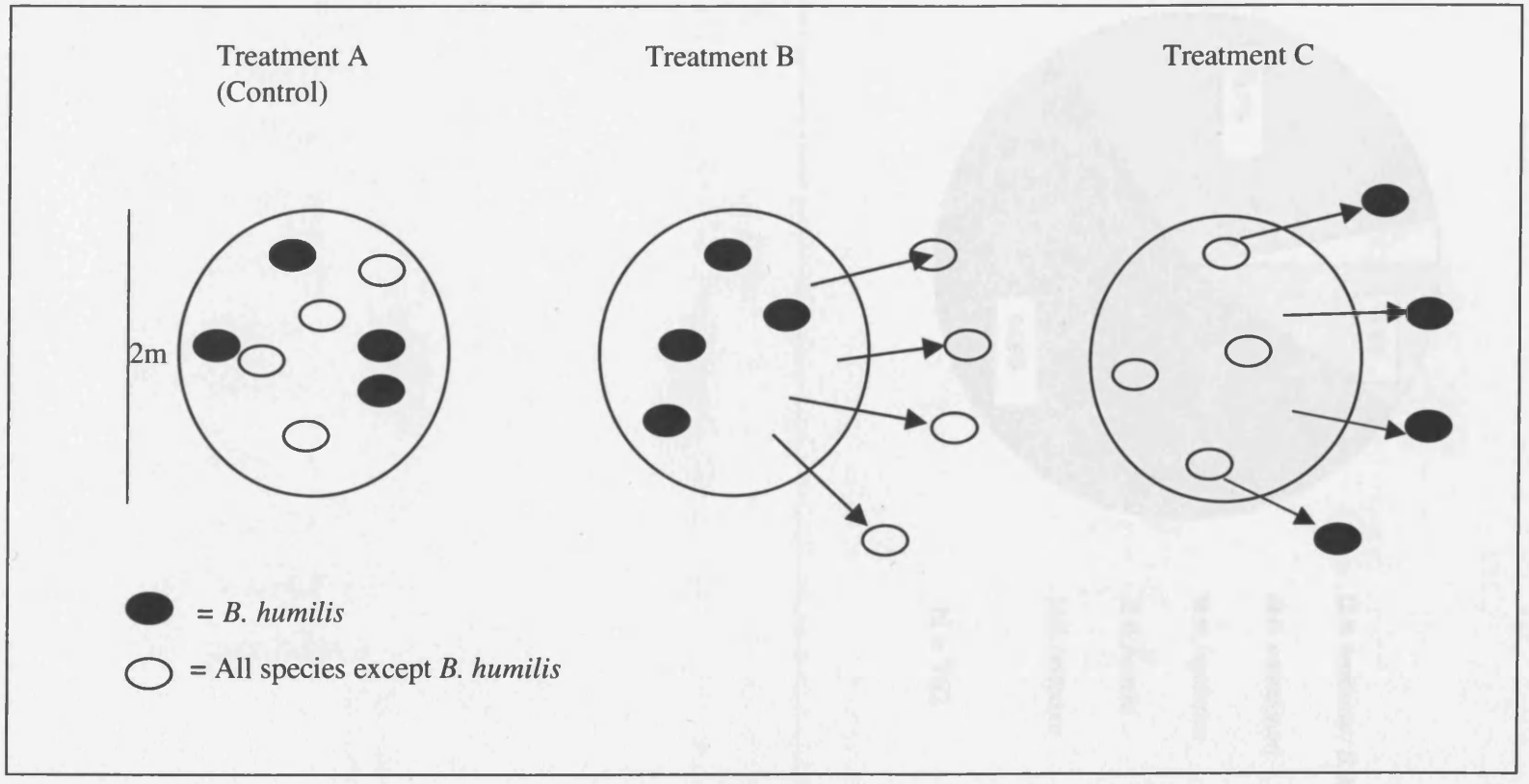


Figure 2. The proportion of species recorded during the 2001 and 2002 study experiments combined.

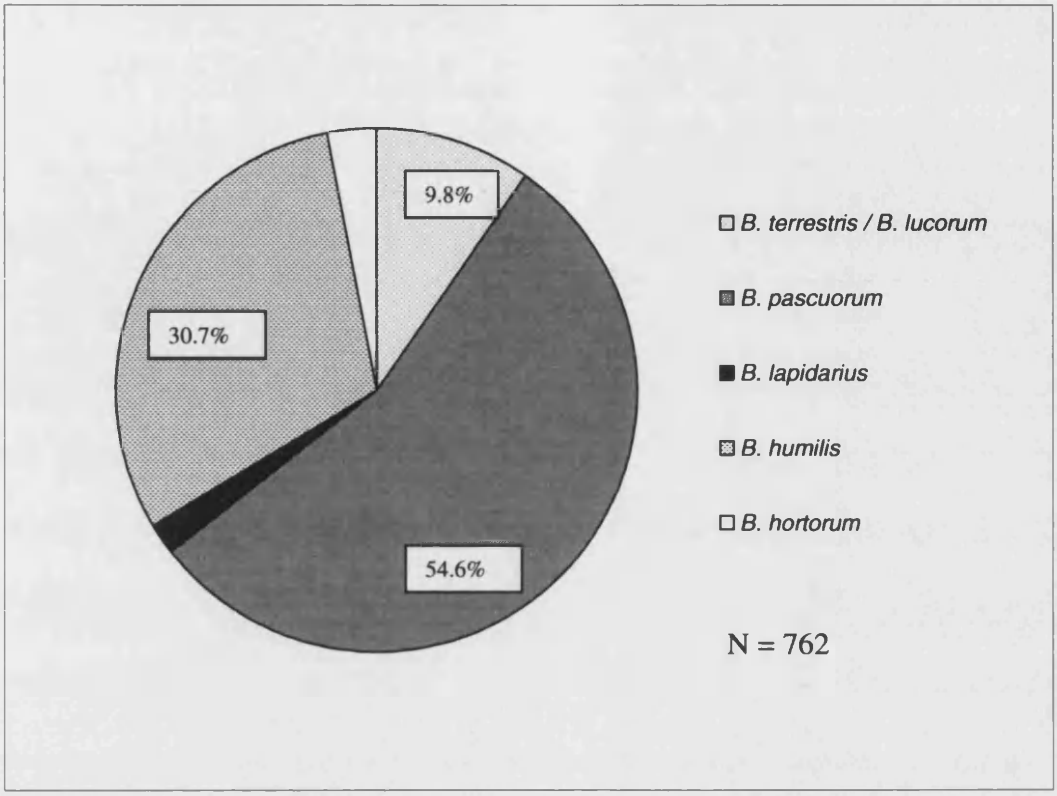


Figure 3a. Results of the 2001 experiment. Treatment A shows the mean time *B. humilis* workers spent foraging in the presence of competitors. Treatment B shows the mean time *B. humilis* workers spent foraging in the absence of competitors. Standard error bars are shown. All times are in seconds.

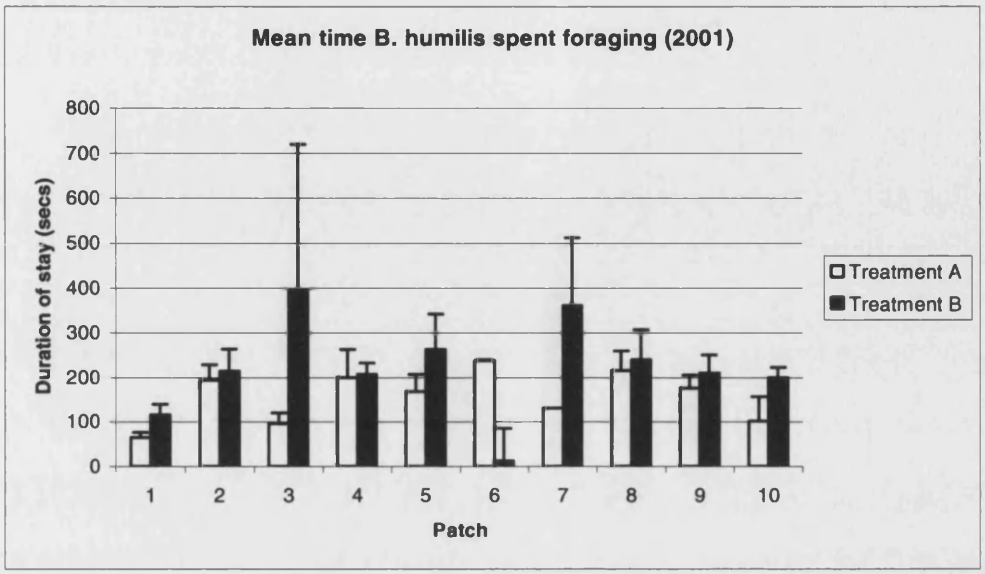


Figure 3b. Results of the 2002 experiment (comparison of treatments A and B). Treatment A shows the mean time *B. humilis* workers spent foraging in the presence of competitors. Treatment B shows the mean time *B. humilis* workers spent foraging in the absence of competitors. Standard error bars are shown. All times are in seconds.

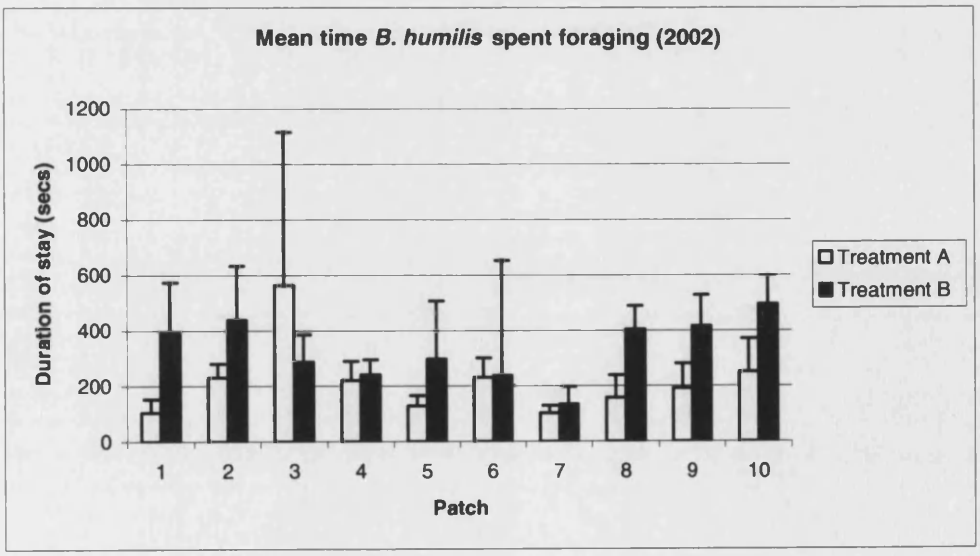


Figure 3c. Results of the 2002 experiment (comparison of treatments A and C). Treatment A shows the mean time workers of other species spent foraging in the presence *B. humilis* workers. Treatment C shows the mean time workers of other species spent foraging in the absence of *B. humilis* workers. Standard error bars are shown. All times are in seconds.

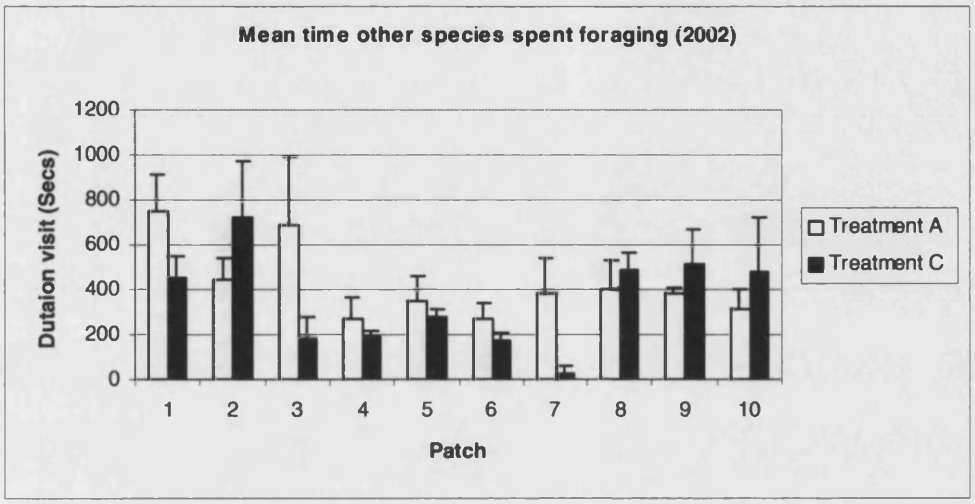


Figure 4a. Mean visit duration of *B. humilis* against the number of competitors of all other species removed (2001).

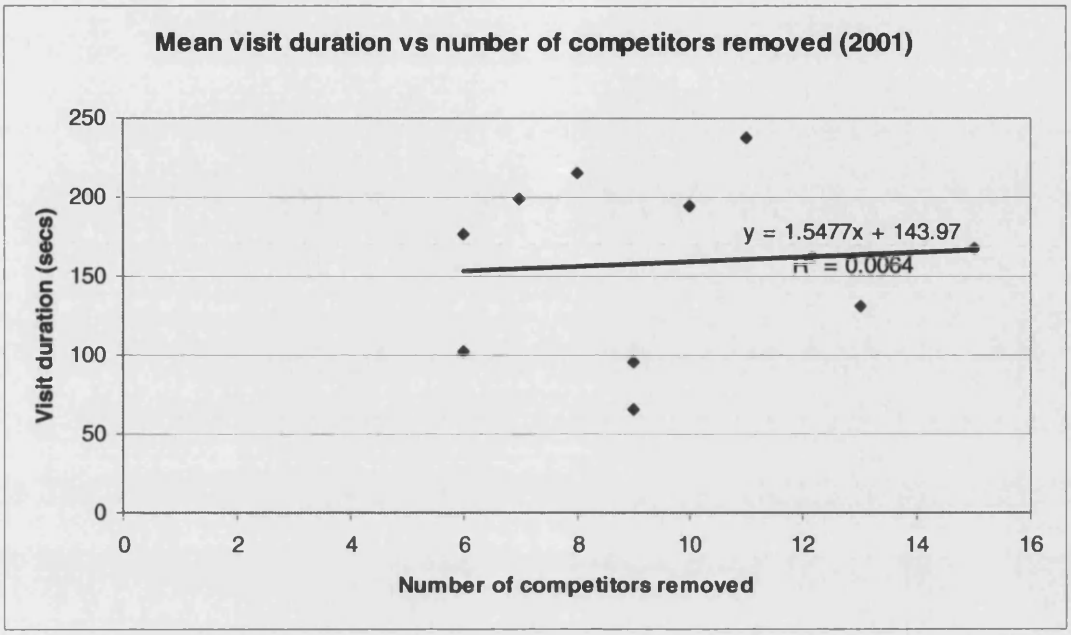
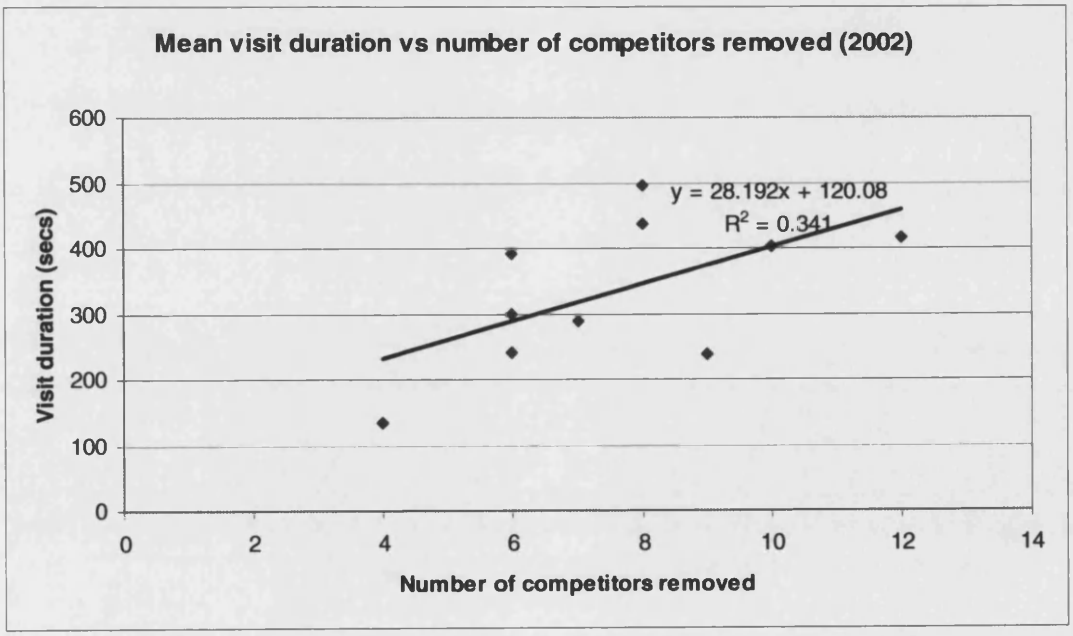


Figure 4b. Mean visit duration of *B. humilis* against the number of competitors of all other species removed (2002).



Chapter 5

Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators

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SUMMARY

Bumble bees play a crucial and irreplaceable role in the pollination of wildflowers and commercial crops. Despite being wild organisms, they are also responsible for the gene flow between many commercial crops, including those that have been genetically modified. The study of such a small and fast moving organism is not easy in the field and as a result there are many questions regarding their foraging ecology that remain unanswered. In this study microsatellite analysis was successfully employed to test three hypotheses concerning the movement patterns of foraging *Bombus* workers at three spatial scales. These hypotheses have not previously been addressed. First, I tested the hypothesis that bumble bee workers from the same colony forage together on patches of flowers; second, I tested whether the number of colonies using an area for forage is small or large; and third, I tested whether there is genetic population differentiation across a large urban landscape. Neither *B. terrestris* nor *B. pascuorum* workers were found to forage with their nest-mates on patches of flowers. In both species a large number of colonies were identified foraging at each site. Mean numbers of 96 and 66 colonies of *B. terrestris* and *B. pascuorum* respectively were identified foraging on sites with a mean area of 0.8 hectares. The number of *B. terrestris* colonies foraging at a site was consistently higher than the number of *B. pascuorum* colonies, with a maximum of 69 colonies of *B. terrestris* being identified foraging over an area of 0.9ha. No inbreeding and little or no genetic differentiation could be detected in either species across the study sites throughout London. As a result of this novel approach to a long-standing and fundamental problem, this work has not only developed a tool to assist in the monitoring and conservation of bumble bees but has also contributed to their evolutionary biology.

1. INTRODUCTION

There are growing concerns over the probable world-wide decline of vertebrate and invertebrate pollinators (e.g. Allen-Wardell *et al.*, 1998; Matheson *et al.*, 1996; Washitani, 1996). Symptoms of pollinator scarcity include reduced fruit and seed set and reduction in yields. Despite the severity of these consequences there are significant gaps in our knowledge of the interactions between invertebrate pollinators and their forage (Kearns & Inouye, 1997). Bumble bees are important as managed and incidental pollinators of commercial crops as well as pollinators of native plants (Corbet *et al.*, 1991) and are known to be particularly important pollinators of leguminous seed crops and fruit trees (Fussell & Corbet, 1991; Stoddard & Bond, 1987). Of the 18 *Bombus* species recorded in Britain since 1900, two are thought to have been lost from Britain in the last fifty years (Williams, 1982, 1986), the most recent, *B. subterraneus* being declared extinct in 1998 (WWF, 1998). Such is the concern over these declines and extinctions that five *Bombus* species are included in the U.K. Biodiversity Action Plan.

Understanding the movement of pollinators over natural and man-made landscapes is particularly important given the current debate over the consequences of the dispersal of pollen, and subsequent gene flow between genetically modified crops and non-genetically modified crops and wild populations of plants (Hails, 2002). Studies of pollinator movement fall into three main categories; those of foraging range, those of individuals' movement between patches of flowers (Thomson, 1996; Thomson *et al.*, 1997; Williams & Thomson, 1998) and those of movement between individual flowers within patches (Saville *et al.*, 1997). Many experimental studies of pollinator

movements have been conducted over small scales, i.e. at patch level (Cartar & Real, 1997; Cresswell, 1997; Cresswell *et al.*, 2000). Foraging bumble bees have been shown to employ a non-random search pattern to maximise their rewards in an environment where resources are patchy and constantly changing. Regular traplining along a route allows an individual to avoid revisiting a flower before it has had time to replenish and constantly monitor resource levels (Thomson, 1981, 1996; Thomson *et al.*, 1987; Thomson *et al.*, 1997; Williams & Thomson, 1998). Because many insect pollinators, including bumble bees, are thought to fly over kilometres to forage, their movements must also be understood at large scales. The study of such a small and fast moving organism at large scales is not easy and in a recent study to model gene flow for an insect-pollinated plant one of the main problems highlighted was that of quantifying the frequency of inter-plant population movements by the pollinators (Cresswell *et al.*, 2002). Foraging bumble bees are notoriously difficult to track and, due to their size, do not lend themselves to the conventional methods used to track individuals of other species. Therefore, fundamental questions regarding their foraging ecology remain unanswered. For example, the distance over which workers will travel to forage has been subject to a great deal of discussion and is acknowledged to be poorly understood (Cresswell *et al.*, 2000). Recent studies point to the fact that bumble bee workers are able to forage efficiently further from the nest than previously thought. It has been shown that, following displacement, bumble bees are capable of flying home from distances of up to 9.8km over several days, although it is not thought that they actually forage over this distance (Goulson & Stout, 2001). As central place foragers, bumble bees lend themselves to theoretical models evaluating their maximum economic range. For example, Cresswell *et al.* (2000)

estimated that the upper limit to a bumble bee's flight range is approximately 10km but concluded that flight ranges of the order of several kilometres are economically viable. It has also been suggested that workers actually avoid foraging close to their nests and concentrate on patches between 360m and 600m from the nest, thereby reducing the risk of attracting predators and parasites to the nest site even though there may be an abundance of forage in the vicinity (Dramstad, 1996). As with many aspects of their ecology, not all *Bombus* species can be considered the same. In a recent study using capture-mark-recapture techniques it was found that the three study species differed in their spatial use of forage, with 69% of resighted *B. muscorum* workers foraging within 100m of their nest (Walther-Hellwig & Frankl, 2000a, b). *B. terrestris* was the widest ranging with 60% of the recaptured workers found foraging between 501m and 1750m from the nest (Hellwig & Frankl, 2000). A technique using harmonic radar to track individual workers has so far shown the most promise as a tool to definitively answer landscape-scale questions regarding bumble bee foraging and has already provided valuable information on orientation flights and foraging constancy (Osborne *et al.*, 1997, 1999; Riley *et al.*, 1996). Using this technology it has been established that workers regularly fly further than 200m to forage, with an overall range of 70-631m (Osborne *et al.*, 1997). It has also been used to confirm previous findings, (Dramstad, 1996; Heinrich, 1976a; Osborne & Williams, 2001; Saville *et al.*, 1997; Thomson, 1996; Thomson *et al.*, 1987; Thomson *et al.*, 1997; Walther-Hellwig & Frankl, 2000a) that individual workers and colonies of some species, favour repeatedly foraging in the same area over periods of hours, or even days (Osborne & Williams, 2001). This technique currently has its limitations regarding range and the environment over which it can be employed. For example, it

only provides accurate coverage over an altitude range of 3m and a distance range of 700m in a flat environment uncluttered by hedgerows, trees and buildings.

The standard and accepted method for censussing bumble bees is the 'bee walk' (Banaszak, 1980; Dramstad & Fry, 1995; Fussell & Corbet, 1993), which can be used to establish species presence-absence, phenology and forage preference. It does not, however, provide information on population size or density. Due to the nature of bumble bees' life-cycle, the reproductive queen spends all but the early days of spring concealed within the nest and only non-reproductive worker bees are visible outside of the nest for the majority of the season. Attempts have been made to estimate bumble bee worker densities using mark-recapture techniques (Teräs, 1983), and, based on his own data and that of Cumber (1953) Harder (1986b) estimated nesting density for all species present combined to be 2-7 nests per hectare at a suitable site. Following extensive work to estimate the area of forage required by several British species, Edwards (1998) concluded that successful nesting densities are probably in the order of one to two queen producing nests per $1km^2$, but that typical nest density varies between species. Intra-specific nest size variation, coupled with the variation in worker numbers within the nest throughout the season, makes it impossible to devise an accurate correlative method for estimating the number of colonies represented by a number of foraging workers. Ideally, accurate and regular nest counts combined with the systematic marking and tracking of individual workers would yield valuable data. However, the cryptic nature of nests, difficulty accessing land, lack of available micro-technology and the likelihood that bumble bees forage over large distances make this impossible over a large area.

The vast majority of the body of bumble bee literature is based on studies carried out on the fragmented agricultural environment where population declines have been most notable. However, urban areas have the potential to provide a refuge for bumble bees that have suffered from the pressures of agricultural intensification, particularly those experienced in southern Britain. London is a particularly good study area because historically the south east of England had the richest *Bombus* fauna in Great Britain (Williams, 1982, 1986, 1989). In this study we take a new methodological approach to test three hypotheses concerning the movement patterns of foraging *Bombus* workers. These hypotheses have not previously been addressed. First, we test the hypothesis that bumble bee workers from the same colony forage together on patches of flowers; second, we test whether the number of colonies using an area for forage is small or large; and third, we test whether there is genetic population differentiation between sites across a large urban landscape. These hypotheses were tested by examining the pattern of microsatellite variation in *B. terrestris* and *B. pascuorum* at three spatial scales across London. These two species were selected for three reasons. First, a species presence and habitat usage survey carried out in May–September 2000 (Chapter 2 of this thesis) revealed them to be the two most abundant and widespread species across the London area. Second, they occur at opposite ends of the *Bombus* ecological requirements spectrum, *B. terrestris* being short-tongued and with a preference for subterranean nest sites and *B. pascuorum* being long-tongued and a surface-nester (Alford, 1975; Prys-Jones & Corbet, 1991). Third, published microsatellite primers exist for both species (Estoup *et al.*, 1995, 1996).

The justification for testing the hypotheses in this study is as follows. Goulson *et al* (1998a, 1998b) have shown that foraging bumble bees workers can detect scent, probably secreted from the tarsus, deposited on flowers by previous bumble bee visitors and thus avoid flowers that have been recently depleted of nectar. It has been shown that this signal is successfully used by conspecific and heterospecific foragers (Stout *et al.*, 1998), but it has never been shown whether nest mates actively forage together thereby also taking advantage of the signal. This study tests if this signal is specifically utilised by nestmates by measuring the genetic relatedness of workers successively visiting the same patch of flowers.

Eusocial insects present a unique problem to conservation biologists in that counting individuals provides very little information about the size of a population and its reproductive potential (Chapman & Bourke, 2001). To my knowledge no previous attempts have been made to systematically quantify the number of wild bumble bee colonies utilising an area for forage.

Previous studies of genetic population structure have explored broad-scale (continental-wide) genetic differentiation. Estoup *et al.* (1996) found no such differentiation among populations of *B. terrestris* from mainland continental Europe. Significant genetic differentiation was identified among *B. pascuorum* populations from continental Europe and it was suggested that the Alps act as a barrier between two isolated gene pools (Widmer & Schmid-Hempel, 1999). On a smaller scale, studies of the genetic structure of island populations have been conducted on *B.*

terrestris from the Canary Islands and Madeira, which found extensive genetic differentiation among islands and between islands and the mainland (Widmer *et al.*, 1998). Similarly, preliminary studies of *B. distinguendus* from Hebridean islands found a certain degree of differentiation (Bourke & Hammond, 2002). The current study represents the first time such an investigation has been carried out on an urban population and on a mainland population at a landscape scale. It has revealed that, despite the landscape being highly fragmented, there appears to be no genetic partitioning between bumble bees occupying areas of 'green space' across London.

2. METHODS

Microsatellites are regions of a genome consisting of short sequences of DNA bases (nucleotides) repeated several times. Genetic variation at any single microsatellite locus (position on a chromosome) is provided by variation in the number of repeats of the sequence. The number of such variants (alleles) at a locus in a population is often high. Furthermore, microsatellite alleles are inherited in a Mendelian fashion and are selectively neutral, unlike a feature such as coat colour, which may be adaptive. Because of their variability, simple mode of inheritance, neutrality and relative ease of typing, microsatellites are the genetic marker of choice for studies of between-individual and between-population genetic variation (Queller *et al.*, 1993). Several polymorphic microsatellite loci in *B. terrestris* and *B. pascuorum* have been identified and employed effectively in previous work (Estoup *et al.*, 1995, 1996; Schmid-Hempel & Schmid-Hempel, 2000; Widmer & Schmid-Hempel, 1999; Widmer *et al.*, 1998).

2.1 Sample collection

Sampling for genetic work was carried out on *B. terrestris* and *B. pascuorum* workers in June and July 2001. Collection sites were selected to be at least 5km apart (mean distance apart = 14.7km) and to be accessible. They were also selected because they attracted relatively large numbers of workers of the target species. A total of 531 *B. terrestris* and 458 *B. pascuorum* workers were collected from the London area for genetic analysis.

Up to 100 workers from each species were collected from five sites in the case of *B. terrestris* and four sites for *B. pascuorum* (mean collection area = 0.8 ha) (Table 1; Figure 1). At each site ten patches of flowers were identified where each patch measured approximately $0.75m^2$ in size and was at least 10m in distance from any other patch (mean distance between patches = 57.4m). The first ten conspecific workers to arrive were collected from each patch and the time between the capture of each bee recorded. A further six sites were selected from each of which ten workers of each species were collected at a minimum distance of 10m apart along a 100m transect (mean collection area = 0.22 ha) (Table 1). In addition, workers were collected directly from three naturally occurring nests and genotyped. This was to check the accuracy of the genotyping of the main samples. Two *B. terrestris* nests were sampled (N = 8, N = 6) and one *B. pascuorum* nest (N = 10) (Appendix C). The first *B. terrestris* nest sampled (N=8) was located in Brompton Cemetery, London (SW12). The second *B. terrestris* nest (N=6) and the *B. pascuorum* nest were located in a private garden in Finchley, London (N3) where colonies are relocated to when they have had to be removed by the council's pest control unit. To ensure that only the target species and caste were sampled during the main collections, each bee was carefully inspected upon or after capture. Unlike those of continental Europe, British *B. terrestris* (ssp. *audax*) workers can be distinguished from those of the otherwise similar *B. lucorum* by either a buff tail or a distinct buff line adjacent to the fourth abdominal tergite (T_4), between the white tip of the 'tail' and the neighbouring black abdominal band (Prys-Jones & Corbet, 1991). *B. pascuorum* is the only common brown bumble bee in the London area. Nevertheless each individual was identified by the presence of black hairs on the thorax to avoid confusion with the similar *B.*

humilis, which has a limited distribution in the London area (Benton, 2000; Chapter 3 of this thesis). Each worker was caught in a plastic (Universal) tube and labelled with an individual identification number. The tubes were placed in a 'cool box' until they could be transferred to a freezer at –80 °C where they remained until the DNA extraction process.

2.2 Genetic Methods

DNA was extracted from an entire middle leg using proteinase K digestion in 1× TE buffer (500µl TE + 10µl of 20mg/mL proteinase K) at 55 °C overnight and then heated to 99°C for 10min to denature the proteinase K prior to the polymerase chain reaction (PCR) phase. All individuals were genotyped at up to six polymorphic microsatellite loci (Estoup *et al.*, 1995, 1996) (Table 2). Different loci were used for the two species because *B. pascuroum* was found to be either fixed or to exhibit too little variation at loci B10, B11 and B100.

PCR amplifications were carried out in a total volume of 20µl with 5µl of genomic DNA. Loci were amplified using one of two reaction conditions, A or B (Table 2). Reaction condition A consisted of: 200nM of each dNTP, 1.5mM $MgCl_2$, 150nM of each primer and 0.5 units of Taq (Gibco); and reaction condition B consisted of: 300nM of each dNTP, 2.5mM $MgCl_2$, 250nM of each primer and 0.5 units of Taq (Gibco). Amplification was performed using a single 'touchdown' thermal profile for all loci (Morin *et al.*, 1998) using the following conditions. All reactions were initially denatured at 95°C for 5 minutes followed by 19 cycles of 95°C for 30s, 65°C for 30s, with the annealing temperature decreasing by 1°C per cycle and an extension

period at 72°C for 30s. This was followed by a further 15 cycles at 95°C for 30s, 45°C for 30s and 72°C for 30s and a final extension period of 7 minutes at 72°C. PCR amplification products were visualised using fluorescent dyes on an ABI PRISM™ 373 automated sequencer and allele sizes were scored with reference to an internal size standard (GeneScanTAMRA 500, Applied Biosystems) using ABI GENESCAN analysis software (Version 3.1) and GENOTYPER DNA fragment analysis software (version 2.0).

A random sample of genotypes across the six loci was retyped to establish a genotyping error rate. In the case of *B. terrestris*, 351 genotypes from 216 workers were retyped at least once, and of these 218 were initially found to be heterozygous and 133 homozygous. Two hundred and eight of 218 heterozygous genotypes were confirmed on retyping (95%) and 102 of 133 homozygous genotypes were confirmed on retyping (77%), with the remaining ones proving to be heterozygotes. In the case of *B. pascuorum*, 383 genotypes from 247 workers were retyped at least once, and of these 255 were initially found to be heterozygous and 128 homozygous. All of the heterozygous genotypes were confirmed on retyping (100%) and 120 of 128 homozygous genotypes were confirmed on retyping (94%), with the remaining ones proving to be heterozygotes. This clearly showed that in both species the main cause of error was allelic drop-out (the non-amplification of an allele). These errors were unlikely to have biased the results in *B. terrestris*, with the highest frequency of allelic drop-outs, for three reasons. First, only a minority of *B. terrestris* genotypes were homozygous meaning that the overall contribution to the error rate from erroneous homozygotes was 8.0%. Second, relatedness, F_{ST} and colony number estimates for the

site with the most (56%) errors (Nunhead Cemetery) were qualitatively identical to those of the site with no such errors (Regent's Park). Third, simulations showed that these errors actually caused colony number to be underestimated from our datasets (see below).

2.3 Data analysis

The amount of genetic linkage, levels of between-site genetic variation (F_{ST}) and levels of inbreeding were calculated using GENEPOP Version 3.1b (Raymond & Rousset, 1995) (web version at http://wbiomed.curtin.edu.au/genepop/) and FSTAT Version 2.9.3.2 (Goudet, 1995), (web version at http://unil.ch/izea/softwares/fstat.html). Tests for linkage disequilibrium and for the presence of inbreeding, if any, were carried out on sub-samples of all of the workers collected from each site. These sub-samples were selected using a random number generator and consisted of groups of 20 bees resampled ten times from the total sample. This sub-sampling procedure was carried out because of the presence in the entire sample of full sisters, which would not represent independent data points owing to the fact that their genotypes might be similar or identical. Pairwise F_{ST} was also calculated using a sub-sample of all of the workers collected. In the case of the six sites from which only 10 workers were collected, all 10 workers were included in the analysis. In the case of the sites from which approximately 100 workers were collected, 10 workers were randomly selected from each site and included in the analysis. A regression analysis of F_{ST} vs. geographical distance was made to test for the correlation between genetic population differentiation and geographical distance using FSTAT. A Mantel test with 20000 permutations was used to test the

significance of the correlation. The program Relatedness 5.0.8 (http://gsoft.smu.edu/Gsoft.html) (Queller & Goodnight, 1989) was used to calculate regression relatedness within the groups of ten workers collected from the same patches of flowers. Where appropriate, the sequential Bonferroni technique (Rice, 1989) was applied to correct for multiple tests and control for Type I error.

Bumble bee species have a single reproductive queen per colony (Alford, 1975). Investigations carried out to establish mating frequencies of *Bombus* species have concluded that all species (including *B. terrestris* and *B. pascuorum*) except for *B. hypnorum* mate with one male (Estoup *et al.*, 1995; Schmid-Hempel & Schmid-Hempel, 2000). All female offspring of a single, once-mated queen will be full sisters and at all loci will possess one of two possible alleles from their mother (maternal alleles) and one allele from their haploid father (paternal allele). Using this rationale the number of full sisterhoods, and thus the number of colonies, in a sample of workers collected from a patch, can be estimated. Using these allele-sharing criteria for haplodiploid full sisters, a program (COLONY 1.0) was developed by Jinliang Wang of the Institute of Zoology. The maximum likelihood sibship reconstruction method Thomas and Hill (2000) was adapted to reconstruct worker sibships from each site at which more than 10 workers were collected (Table 1). To verify the method's power, simulations were run for the case of 100 individuals typed at 6 loci, each having 10 co-dominant alleles following a uniform frequency distribution. Allelic drop-out rates of (i) 0% and (ii) 20% per locus were assumed. For (i), where the 'actual' colony size followed a truncated Poisson distribution with $m = 0.9$ and $m = 15$ (where m is the mean size of the workforce representing each colony in the sample),

the estimated numbers of colonies were 0.92 ± 0.03 and 1.0 ± 0.0 (mean ± SD, n = 100 replicates) of their actual values, respectively. Therefore the method's power increased with increasing m, but, for the case of m = 0.9, which mimics our data (i.e., many colonies represented at each site sampled by a few workers each; see Results), it slightly underestimates the colony number. This is because the number of unrelated individuals that, by chance, have multi-locus genotypes consistent with full sisterhood increases as the colony number increases. For (ii) with m = 0.9, a more conservative estimate was obtained (0.85 ± 0.04 of the actual colony number, n = 100 replicates) than for (i). Therefore the observed levels of allelic drop-out in our dataset caused an additional underestimate of colony number. For larger families (m>>0.9), allelic drop-out resulted in overestimates of colony number because larger families were split. The effects of other types of error in the genetic data, such as scoring and data entry, were not explored. Although such errors might have been present in the data they were likely to have been rare because, of 139 genotypes scored from 24 additional workers collected from the three known nests, the genotype of only one worker was inconsistent with monogyny and monandry and hence likely to have been a scoring or data entry error. When calculating the average number of *B. terrestris* colonies visiting sites, the Regent's Park site was excluded in order to equalize the sample sizes (98-100 workers) of included sites (Table 1).

3. RESULTS

3.1 Genetic linkage, genetic variation and inbreeding

A total of 531 and 458 workers were typed at an average of 5.8 (range 3-6) loci each for *B. terrestris* and *B. pascuorum* respectively (Table 2; Appendix D; Appendix E). All loci showed high levels of polymorphism. The average number of alleles per locus was 10.3 (range 6-15) for *B. terrestris* and 8.8 (range 6-12) for *B. pascuorum* (Table 3). The average observed heterozygosity across study sites was 65.5% (range 57.3-70.3) and 67% (range 64.2-68.8) for *B. terrestris* and *B. pascuorum* respectively (Table 3).

No evidence was found for linkage disequilibrium between loci in either species. To test for non-random associations between pairs of loci, 10 × 15 possible pairwise comparisons between each of the six loci were carried out on 10 sub-samples of 20 individuals randomly selected (using a random number generator) from the entire sample of 531 *B. terrestris* and 458 *B. pascuorum* workers. Only three and 12 comparisons for *B. terrestris* and *B. pascuorum* respectively gave significant values ($P < 0.05$) and no comparisons tested significant after Bonferroni correction for multiple tests. This suggests that the six loci used for *B. terrestris* and the six used for *B. pascuorum* represent independent genetic markers.

The average Weir and Cockerham's inbreeding coefficient (F_{IS}) over the six loci ranged from –0.025 in Regent's Park to 0.115 in Barnes Common for *B. terrestris* and –0.003 in Millennium Village to 0.074 in Barnes Common for *B. pascuorum* (Table 4). No evidence was found for significant inbreeding at any site. In the case of *B.*

terrestris, the global $\underline{F}_{IS}$ value was 0.062 (N = 5 sites, all $P > 0.14$); and, in *B. pascuorum,* the global $\underline{F}_{IS}$ value was 0.052 (N = 4 sites, all $P > 0.14$). These findings concur with work conducted on continental and island populations of *B. terrestris* (Estoup et al., 1996), commercially bred *B. terrestris* (Lopez-Vaamonde *et al.*, 2003) and *B. distinguendus* (Bourke & Hammond, 2002).

3.2 Within-flower patch relatedness

The mean time over which ten workers were collected was 41 and 31 minutes for *B. terrestris* and *B. pascuorum* respectively (Table 8). For *B. terrestris,* mean relatedness (R) of workers within patches was 0.008 (range = -0.065 – 0.216, N = 50 patches of mean 9.4 workers each), where R = 0.75 would indicate that workers within patches were outbred full sisters. No patch had worker-worker relatedness significantly greater than zero at any site (all $P > 0.001$, corresponding to table-wide $P = 0.05$) and all patches had worker-worker relatedness significantly less than 0.75 (Table 5a). For *B. pascuorum,* mean within-patch relatedness was 0.012 (range = -0.068 – 0.147, N = 40 patches of mean 9.9 workers each); no patch had worker-worker relatedness significantly greater than zero at any site (all $P > 0.0012$, corresponding to table-wide $P = 0.05$) and all patches had worker-worker relatedness significantly less than 0.75 (Table 5b). As a control, workers from known nests were subjected to the analysis described above and tested against 0.75. Two *B. terrestris* nests were sampled. In the case of nest one, R ± SE = 0.89 ± 0.06 (d.f. = 7, $P = 0.07$) and, in the case of nest two, R = 0.90 ± 0.06, (d.f. = 5, $P = 0.05$). For the single *B. pascuorum* nest analysed, R ± SE = 0.93 ± 0.04 (d.f. = 9, $P = 0.04$). In all three cases, R is larger than the 0.75 value expected for full sisters, and significantly so in the case of the *B. pascuorum*

nest. This can be attributed to the small sample size, which would fail to sample the full range of allelic variation. Furthermore, in the case of the *B. pascuorum*, nest relatedness is particularly high because, although we did not know it at the time, one of the six loci used, B100, is fixed, and two loci, B10 and B11, show very little allelic variation in this species for this population, thereby reducing the power of the analysis. When the analysis was re-run on the *B. pascuorum* nest using the three loci with sufficient variation, R ± SE = 0.81 ± 0.01 (d.f. = 9, $P = 0.003$). This value was lower than the previous estimate but still significantly greater than 0.75 because of the smaller standard error. Having identified this problem, three further loci were selected, B118, B131 and B132, which were shown to exhibit greater allelic diversity, and used for the remainder of the genotyping of the *B. pascuorum* samples. This analysis of individuals from known nests showed genotypes consistent with the queen being singly-mated, although there was one anomalous genotype (N genotypes = 139), which was likely to have been due to a scoring or data entry error.

3.3 Number of colonies per site

More *B. terrestris* colonies were found to be visiting sites than *B. pascuorum* colonies at all sites (Table 6). The mean minimum number of *B. terrestris* colonies identified per site was 63 (n = 4 sites with 99.2 workers genotypes per site) and the mean minimum number of *B. pascuorum* colonies identified per site was 52 (n = 4 sites with 99.5 workers genotypes per site) (Table 6). The greatest number of *B. terrestris* colonies was sampled from Thames Barrier Park (69) and the least from Nunhead Cemetery (58). Barnes Common produced the most *B. pascuorum* (60) and Millennium Village the least (40). These estimates are conservative for two reasons.

First many colonies are represented in each site by a few workers each and the number of unrelated individuals that, by chance, have multilocus genotypes consistent with full sisterhood increases as colony number increases. Second, many of the colonies visiting the site will not have been sampled. To investigate the scale of this under-sampling, the datasets from all sites within each species were pooled. The observed frequency distribution of the sizes of the workforces from different colonies present in the entire sample did not differ significantly from a truncated Poisson distribution (*B. terrestris*: $\chi^2 = 6.0$, d.f. = 3; *B. pascuorum*: $\chi^2 = 10.4$, d.f. = 5, both $P > 0.05$). I therefore used fitted Poisson distributions to estimate the frequency of colonies not represented in the sample and therefore the average overall number of colonies visiting the site. This increased the overall average (95% confidence limits) to 96 colonies (84-118) per site for *B. terrestris* and 66 colonies (61-76) for *B. pascuorum.*

3.4 Genetic differentiation

There was no significant genetic differentiation between populations of *B. terrestris* and slight but significant differentiation between populations of *B. pascuorum* (*B. terrestris*: global F_{st} [95% confidence limits] = 0.000 [–0.012 to 0.012]; *B. pascuorum*: global F_{st} [95% confidence limits] = 0.009 [0.003 to 0.015]). This suggests that gene flow between sites is not limited in *B. terrestris* and only slightly limited in *B. pascuorum.* Pairwise F_{st} varied from a maximum of 0.046 (between Thames Barrier and St. James' Lane) to –0.0003 (between Thames Barrier and Nunhead Cemetery) in *B. terrestris* (Table 7a) and from a maximum of 0.039 (between Regent's Park and Hanwell) to –0.007 (between Barnes Common and

Tolworth) in *B. pascuorum* (Table 7b). Randomisation tests showed that there was significant pair-wise differentiation between none of 55 pairs of populations in *B. terrestris* (Table 7a) and between only one of forty five pairs of populations in *B. pascuorum,* (Hanwell and Regent's Park populations) (Table 7b). There was no significant relationship between the degree of genetic differentiation among the sites and the geographical distance between the sites in either species (Mantel tests: *B. terrestris* $r^2 = 6.52\%$; $P = 0.058$; *B. pascuorum* $r^2 = 0.1\%$; $P = 0.877$).

4. DISCUSSION

This study shows that microsatellite analysis can be successfully employed to explore aspects of bumble bee foraging ecology at different spatial scales. Neither *B. terrestris* nor *B. pascuorum* workers were found to forage preferentially with their nest-mates on patches of flowers. The estimated number of *B. terrestris* colonies foraging at a site was consistently higher than the number of *B. pascuorum* colonies. The mean minimum number of *B. terrestris* colonies identified per site was 63 (n = 4 sites with 99.2 workers genotypes per site) and the mean minimum number of *B. pascuorum* colonies identified per site was 52 (n = 4 sites with 99.5 workers genotypes per site). The most colonies were identified at the Thames Barrier Park, with an estimated 69 colonies of *B. terrestris* identified foraging over an area of 0.88ha. No inbreeding effects were identified and little or no genetic differentiation could be detected in either species across the study sites throughout London.

Goulson *et al.* (1998a, 1998b) observed that, through detecting scent marks, worker bumble bees selectively rejected inflorescences that had been recently visited by themselves, by conspecifics and workers of other *Bombus* species, thus avoiding flowers recently depleted of nectar and improving their foraging efficiency. It is not clear whether the laying down of scent marks is an active process. Kin selection theory (Hamilton, 1964) predicts that selection should not favour the production of costly signals of resource quality benefiting unrelated conspecifics or members of other species. If there is a metabolic cost to producing these secretions and marking the flowers then it follows that it would only be beneficial to signal to oneself or to one's nestmates and therefore we would expect to find bees from the same colony

foraging together. It has been shown in this study that bumble bees from the same nest do not forage together but instead workers foraging in succession on one patch of flowers are, on average, from different colonies. These findings concur with those of a recent study that shows that although there is within-nest communication to indicate the presence of quality forage and the scent of that forage in bumble bees, there is no evidence for communication regarding the location of that forage (Dornhaus & Chittka, 1999). In addition, it is thought that workers range too far and colony densities are too high for workers to encounter siblings regularly (Thomson & Chittka, 2001). Therefore it is difficult to explain the evolution of the repellent scent mark using kin selection theory. Thomson and Chittka (2001) suggest that tarsal secretions might primarily be used for adherence of bee feet to flowers and that bees have learned to interpret the strength of the scent as an indication of nectar availability. The chemistry of tarsal extracts have been found to be very similar to those of the rest of the body (Goulson *et al.*, 2000). Artificial scent marks, synthesised from pure chemical constituents and applied manually to flowers, have induced a repellent response in foraging bees (Goulson *et al.*, 2000; Stout *et al.*, 1998). With this in mind it is most likely that the repellent scent mark is nonadaptive (Goulson *et al.*, 1998a; Stout *et al.*, 1998; Williams, 1998) and is produced as a cost-free metabolic by-product, deposited inadvertently when the bee comes in contact with the flower. It is also possible that in situations of low resources and high levels of competition there may be a higher level of perception of the scent amongst nestmates and only in that situation would they specifically benefit.

In both species a large number of colonies were identified foraging at each site. Mean numbers of 96 and 66 colonies of *B. terrestris* and *B. pascuorum* respectively were identified foraging on sites with a mean area of 0.8 hectares. It must be noted that the study sites were selected on the basis of having large numbers of workers, so it cannot be assumed that all areas of forage support such a large number of colonies. If one considers the distance over which colonies forage these large numbers are entirely plausible. For example, if bumble bee nests occur at a density of 1-2 nests per $1km^2$ (Edwards, 1998) and 30 colonies are identified at a site, as is the case of *B. terrestris* at the Regent's Park, then the catchment area of that site must be 30 km^2. The radius of a circle with an area of 30 km^2 is 3.1km ($\pi r^2 = 30$, $r^2 = 9.55$, $r = 3.1$). Therefore, at the furthest, a worker would have to fly 3.1km to forage at the study site. The median distance a worker would have to fly is 2.2km ($\sqrt{0.5}$ r) since 50% of the colonies would be further than this distance from the centre of the circle and 50% of the colonies would be less than this distance from it. For the number of colonies to be sampled to remain constant, the distance a worker is estimated to fly increases as the nest density decreases (Figure 2). Foraging ranges estimated from Figure 2 are consistent with those in the literature, as outlined in the introduction to this chapter. For example, in this study, *B. terrestris* have been shown to forage at densities of 58 - 69 colonies over a mean area of 0.8ha (Table 6). Assuming the nesting density estimate of 1-2 nests per km^2 (Edwards, 1998), median foraging distances were calculated to be 1.2-3.0km for *B. terrestris*. This concurs with estimates of, for example, $\leq$ 1.75km (Walther-Hellwig & Frankl, 2000b), several km (Goulson & Stout, 2001), >2km (Dramstad & Fry, 1995) and 3-4km (Hedtke & Schricker, 1996). The fact that consistently fewer *B. pascuorum* colonies are found at each site may be due to their occurring at lower

densities or the fact that, as 'front door foragers', *B. pascuorum* operate on a smaller spatial scale than *B. terrestris* (Hedtke & Schricker, 1996). Initially it was surprising that there was not a greater difference between the maximum and minimum number of *B. pascuorum* colonies sampled. The highest number of *B. pascuorum* colonies was sampled from Barnes Common (60) and the least from Millennium Village (40), which might lead one to believe that Barnes Common is the best habitat for *B. pascuorum.* Barnes Common is situated in a very built up part of London and covers an area of 39.4 hectares of mixed habitat. Millennium Village on the other hand is in the East End of London and is part of a very attractive mosaic of brownfield sites and riverbanks that provide foraging sites and the undisturbed areas of long grass required for nest habitat. Barnes Common may be acting as an oasis for bees in west London, drawing *B. pascuorum* in to forage from greater distance, whereas workers in the East End have a greater choice of forage sites and thus do not have to forage at such high densities. A large proportion of Barnes Common is also undisturbed tussock grassland and could provide suitable nesting habitat for *B. pascuorum,* which, again as 'front door foragers', may be nesting in and foraging on the same large area. The fact that patches of forage are attracting large numbers of colonies to them, over distances of several kilometres, has two main conservation management implications. Firstly, if bumble bees are not nesting and foraging at the same site, these habitats can be considered as separate units when it comes to management strategies. Secondly, it means that any conservation strategies should be planned and executed on an appropriate scale. Rather than just one or two boroughs being pro-active, there needs to be a city-wide effort to enhance the matrix of patches of forage and nesting habitat that already exist.

At this landscape scale, no genetic differentiation was detected between the study sites in *B. terrestris* and very little in *B. pascuorum*. This is unsurprising given that in a recent study microsatellite analysis was unable to identify any differentiation among continental populations of *B. terrestris* (Estoup *et al.*, 1996). Although a previous study carried out to examine the population genetic structure of *B. pascuorum* from continental Europe did identify differentiation among some populations, this was attributed to the presence of the Alps mountain range presenting a partial barrier to gene flow (Widmer & Schmid-Hempel, 1999). Nevertheless, the present study shows that, at this scale, there has been extensive recent or current gene flow among the populations across the sites. This implies that foraging workers fly far and / or queens fly far from their natal colony before founding their colony (Mikkola, 1984; Stenstrom & Bergman, 1998). Clearly the significant barriers posed by the urban environment are not sufficient to prevent the movement of queens and foraging workers, or gene flow, between areas.

In previous studies individual workers have been shown to exhibit high degrees of site fidelity and marked individuals return to the same patch of forage repeatedly over time until the resource becomes unprofitable (Dramstad, 1996; Heinrich, 1976a; Osborne *et al.*, 1999; Osborne & Williams, 2001; Saville *et al.*, 1997). This study has shown that colonies operate on a large spatial scale and workers, in this environment, fly as far as several kilometres to forage. The fact that workers from the same colonies are not found in large numbers on the same patch of flowers, or even at the same site, at any one time, means that workers from the same colonies are likely to be flying to

many sites to forage. Viable pollen can be transferred upon contact between workers within the nest in honey bees (DeGrandi-Hoffman *et al.*, 1986; Free & Williams, 1972) and, in bumble bees, thereby has the potential to travel distances greater than just the flight range of an individual worker. Similarly, the fact that workers from so many colonies have the potential to make contact outside of the nest has significant implications for the transmission of parasites (Schmid-Hempel, 1998).

It is impossible to ascertain from this study whether urban environments provide a better habitat for bumble bees than rural environments, but a recent study has shown that *B. terrestris* nests gained weight more quickly and reached a larger final size in a suburban environment than in both a conventional farmland environment and farmland under schemes to promote biodiversity (Goulson *et al.*, 2002). The fact that so many colonies have been identified foraging at sites does indicate that, in the case of *B. terrestris* and *B. pascuorum* at least, such an environment seems to support healthy populations. The presence of three rare species of British *Bombus*, *B. sylvarum*, *B. humilis* and *B. ruderarius* (Chapter 3, this thesis), adds weight to the argument that the flower-rich green spaces of London may be providing a refuge for bumble bees that suffer from a lack of forage and nest sites in the surrounding countryside. With the ever-increasing demand for housing and commercial development in the city comes pressure to redevelop brownfield sites rather than developing peripheral greenfield sites, thereby weakening the matrix. Obviously there is only a certain amount that can be done to influence what people plant in their private gardens. Where the influence can be exerted is during the planning of new housing developments, which replace valuable brownfield sites, such as the recently

completed Thames Barrier Park and Millennium Village in London's East End. Both sites have included substantial public green space planted with a combination of formal gardens and wildflower seed mix, which have been shown to provide excellent forage at least.

The development of the method described in this chapter has significant conservation management applications such as estimating population density, determining habitat quantity requirements, assessing habitat quality, clarifying conservation status and monitoring population changes. Furthermore, this method is not restricted to bumble bees and can be applied to other organisms too. Now that a protocol for the non-lethal sampling DNA from bumble bees has been established (Holehouse *et al.*, 2003), this method can be applied to rarer species such as *B. humilis*, *B. sylvarum* and *B. distinguendus*, which have conservation status but about which little more is known than the distribution range and forage utilisation. Although this method alone produces the desired results, when combined with ever-improving tracking technology it could become an even more powerful tool to facilitate conservation biologists to prioritise resources, develop and implement management strategies and monitor their effectiveness.

Table 1. Samples of *B. terrestris and B. pascuorum* workers collected from 11 sites across London for genetic analysis. Although the aim was to collect 100 workers at the first five sites listed below, N does not always equal 100. This is mainly due to lost samples, except for Regent's Park where a large number of *B. terrestris* males were accidentally collected. The flowers that *B. terrestris* were collected from were: *Ballota nigra, Campanula* sp., *Centaurea nigra, Cirsium arvense, Deutzia var., Epilobium sp., Hebe* var., *Lavendula* 'Hidcote', *Lotus corniculatus, Rubus fruticosus, Solanum dulcamara, Trifolium pratense* and *Trifolium repens. B. pascuorum* were collected from: *B. nigra, C. nigra, G. officinalis, Lamium album, Lathyrus pratensis, L. corniculatus, R. fruticosus, S. dulcamara, S. nigrum, T. pratense* and *T. repens, and V. cracca.*

Species				***B. terrestris***		***B. pascuorum***	
Site Name	**Grid reference**	**Collection area (ha)**	**Habitat Type**	**N**	**Date collected**	**N**	**Date Collected**
Nunhead Cemetery (SE15)	TQ355755	0.85	Cemetery	100	26.06.01	100	10.07.01
Barnes Common (SW13)	TQ225759	0.91	Public Park	99	03.07.01	100	03.07.01
Regent's Park (NW1)	TQ277833	0.66	Public Park	74	20.07.01	100	20.07.01
Millennium Village (SE10)	TQ399792	0.7	Public Park	100	27.07.01	98	27.07.01
Thames Barrier Park (E16)	TQ412798	0.88	Public Park	98	05.07.01	0	-
Tolworth Roundabout (KT9)	TQ198650	0.30	Brownfield	10	21.06.01	10	03.07.01
Hanwell Cemetery (W7)	TQ159800	0.28	Cemetery	10	21.06.01	10	30.06.01
Woodgrange Park (E12)	TQ418851	0.18	Cemetery	10	22.06.01	10	22.06.01
St. James Lane (N10)	TQ288895	0.14	Garden	10	22.06.01	10	23.07.01
Beddington Park (CR0)	TQ290654	0.28	Public Park	10	25.06.01	10	25.06.01
Grove Park (SE9)	TQ415725	0.14	Public Park	10	26.06.01	10	26.06.01
Total collected				**531**		**458**	

Table 2. Microsatellite loci tested for amplification in *B. terrestris* and *B. pascuorum*. See text for details of PCR conditions A and B.

Species	Locus*	PCR conditions	Total no. of alleles	No. of individuals typed at locus
B. terrestris	B10	B	20	514
B. terrestris	B11	A	14	526
B. terrestris	B96	A	11	509
B. terrestris	B100	A	10	522
B. terrestris	B124	A	18	494
B. terrestris	B126	A	14	507
B. pascuorum	B96	A	9	457
B. pascuorum	B118	B	15	449
B. pascuorum	B124	A	16	452
B. pascuorum	B126	A	9	454
B. pascuorum	B131	B	15	445
B. pascuorum	B132	B	11	382

* (Estoup *et al.*, 1995, 1996)

Table 3. A summary of microsatellite variation in *B. terrestris* and *B. pascuorum,* showing the mean number of alleles per locus (N†), the number of alleles (N_a), observed heterozygosity (H_{obs}) and number of workers genotyped (n) per study site.

Locus		Site														
		Nunhead Cemetery			Barnes Common			Regent's Park			Millennium Village			Thames Barrier		
	N†	N_a	H_{obs}	n	N_a	H_{obs}	n	N_a	H_{obs}	n	N_a	H_{obs}	n	N_a	H_{obs}	n
B. terrestris																
B10	15	13	64	93	18	83	97	14	69	74	16	81	98	14	73	97
B11	9	8	72	100	10	71	97	9	53	74	9	71	99	9	75	96
B96	5.8	5	46	97	5	44	94	5	46	74	7	55	92	7	48	93
B100	8.2	9	66	98	9	58	98	6	63	74	9	69	100	8	69	94
B124	11.6	9	79	98	16	70	94	9	54	65	11	69	85	13	78	98
B126	12	12	79	97	13	67	99	11	59	72	13	57	95	11	79	97
Mean per population	**10.3**	**9.3**	**67.7**	**97.1**	**11.8**	**65.5**	**99.5**	**9**	**57.3**	**72.1**	**10.8**	**67**	**94.8**	**10.3**	**70.3**	**95.8**
B. pascuorum														-	-	-
B96	7	8	65	100	8	84	100	6	72	100	6	74	97	-	-	-
B118	10.25	10	77	95	10	44	63	11	66	75	10	77	95	-	-	-
B124	9.25	7	77	93	10	69	100	10	70	100	10	68	98	-	-	-
B126	6	5	38	99	5	48	100	8	47	99	6	38	98	-	-	-
B131	11.5	11	78	100	10	76	99	11	78	100	14	68	96	-	-	-
B132	8.75	10	78	97	9	64	99	9	71	100	7	82	93	-	-	-
Mean per population	**8.8**	**8.5**	**68.8**	**97.3**	**8.6**	**64.2**	**93.5**	**9.1**	**67.3**	**95.6**	**8.8**	**67.8**	**96.2**	-	-	-

Table 4. A summary of Weir and Cockerham's inbreeding coefficient (F_{IS}) for *B. terrestris* and *B. pascuorum* at all sites, tested for significant difference from zero.

Species	Site	F_{IS}		Mean *P* - value
		Mean	Range	
B. terrestris	Nunhead Cemetery	0.1144	0.0432 - 0.2428	0.2560
B. terrestris	Barnes Common	0.1154	0.0357 - 0.1887	0.2552
B. terrestris	Regent's Park	-0.0251	-0.1808 - 0.0272	0.1436
B. terrestris	Millennium Village	0.1001	0.0443 - 0.2090	0.2163
B. terrestris	Thames Barrier	0.1093	0.0425 - 0.1948	0.2621
B. pascuorum	Nunhead Cemetery	0.0459	-0.0422 - 0.1145	0.4089
B. pascuorum	Barnes Common	0.0744	0.0158 - 0.1728	0.2761
B. pascuorum	Regent's Park	0.0669	0.0025 - 0.1380	0.2971
B. pascuorum	Millennium Village	-0.0028	-0.0972 - 0.0755	0.2823

Table 5a. *B. terrestris* within-patch worker-worker relatedness where R value = regression relatedness, n = number of workers in patch sample, SE = standard error. t-tests were conducted to test whether R was significantly different from zero (t_1) and 0.75 (t_2). Mean number of workers in patch sample (n) = 9.42.

Site	Patch no.	R value	n	S.E.	t_1	*P*	t_2	*P*
Nunhead Cemetery	1	0.0291	10	0.0244	1.1926	0.2635	29.5451	0.0000
	2	-0.0098	10	0.0474	0.2068	0.8408	16.0295	0.0000
	3	-0.0066	10	0.0362	0.1823	0.8594	20.9006	0.0000
	4	0.0138	10	0.0119	1.1597	0.2760	61.8655	0.0000
	5	0.104	10	0.0352	2.9545	0.0161	18.3523	0.0000
	6	-0.0137	10	0.0365	0.3753	0.7161	20.9233	0.0000
	7	-0.0198	10	0.0301	0.6578	0.5271	25.5748	0.0000
	8	0.0163	10	0.0193	0.8446	0.4202	38.0155	0.0000
	9	-0.0319	10	0.0301	1.0598	0.3168	25.9767	0.0000
	10	0.0093	10	0.0302	0.3079	0.7651	24.5265	0.0000
Barnes Common	11	-0.0128	10	0.0297	0.4310	0.6766	25.6835	0.0000
	12	-0.0194	10	0.0317	0.6120	0.5557	24.2713	0.0000
	13	0.0039	10	0.0315	0.1238	0.9042	23.6857	0.0000
	14	0.0704	10	0.0289	2.4360	0.0376	23.5156	0.0000
	15	-0.0286	10	0.0158	1.8101	0.1037	49.2785	0.0000
	16	0.0181	10	0.0392	0.4617	0.6552	18.6709	0.0000
	17	0.0255	10	0.0409	0.6235	0.5484	17.7139	0.0000
	18	0.0796	10	0.0477	1.6688	0.1295	14.0545	0.0000
	19	-0.0101	10	0.0356	0.2837	0.7831	21.3511	0.0000
	20	-0.0411	9	0.0351	1.1709	0.2753	22.5385	0.0000
Regent's Park	21	0.2161	7	0.0502	4.3048	0.0051	10.6355	0.0000
	22	-0.0649	8	0.018	3.6056	0.0087	45.2722	0.0000
	23	-0.0603	6	0.0502	1.2012	0.2835	16.1414	0.0000
	24	-0.0087	8	0.0447	0.1946	0.8512	16.9732	0.0000
	25	0.0088	8	0.0239	0.3682	0.7236	31.0126	0.0000
	26	0.0071	7	0.0721	0.0985	0.9248	10.3037	0.0000
	27	-0.0319	4	0.0707	0.4512	0.6825	11.0594	0.0000
	28	0.0962	6	0.0815	1.1804	0.2909	8.0221	0.0005
	29	0.0653	10	0.0249	2.6225	0.0277	27.4980	0.0000
	30	0.0025	10	0.0346	0.0723	0.9440	21.6040	0.0000
Millennium Village	31	0.0162	10	0.0355	0.4563	0.6590	20.6704	0.0000
	32	-0.0353	10	0.0169	2.0888	0.0663	46.4675	0.0000
	33	0.0052	10	0.023	0.2261	0.8262	32.3826	0.0000
	34	0.022	10	0.0524	0.4198	0.6844	13.8931	0.0000
	35	-0.0365	10	0.0234	1.5598	0.1532	33.6111	0.0000
	36	-0.0028	10	0.037	0.0757	0.9413	20.3459	0.0000
	37	-0.0022	10	0.0536	0.0410	0.9682	14.0336	0.0000
	38	-0.0366	10	0.0458	0.7991	0.4448	17.1747	0.0000
	39	-0.0426	10	0.028	1.5214	0.1625	28.3071	0.0000
	40	0.045	10	0.0315	1.4286	0.1869	22.3810	0.0000
Thames Barrier Park	41	-0.0133	10	0.0291	0.4570	0.6585	26.2302	0.0000
	42	0.017	10	0.04	0.4250	0.6808	18.3250	0.0000
	43	0.0183	10	0.05	0.3660	0.7228	14.6340	0.0000
	44	-0.0256	10	0.0325	0.7877	0.4511	23.8646	0.0000
	45	0.1031	10	0.067	1.5388	0.1582	9.6552	0.0000
	46	-0.0365	9	0.0135	2.7037	0.0269	58.2593	0.0000
	47	-0.0543	10	0.0336	1.6161	0.1405	23.9375	0.0000
	48	-0.029	9	0.0453	0.6402	0.5400	17.1965	0.0000
	49	0.0308	10	0.0432	0.7130	0.4939	16.6481	0.0000
	50	0.0632	10	0.04	1.5800	0.1486	17.1700	0.0000

Table 5b. *B. pascuorum* within-patch worker-worker relatedness, where R value = regression relatedness, n = number of workers in patch sample, SE = standard error. t-tests were conducted to test whether R was significantly different from zero (t_1) and 0.75 (t_2). Mean number of workers in patch sample (n) = 9.95.

Site	Patch No.	R value	n	S.E.	t_1	*P*	t_2	*P*
Barnes Common	1	0.0992	10	0.0578	1.7163	0.1202	11.2595	0.0000
	2	-0.0094	10	0.0267	0.3521	0.7329	28.4419	0.0000
	3	0.0373	10	0.071	0.5254	0.612	10.0380	0.0000
	4	-0.0273	10	0.0779	0.3504	0.7341	9.9782	0.0000
	5	0.0789	10	0.0851	0.9271	0.378	7.8860	0.0000
	6	0.0321	10	0.0474	0.6772	0.5153	15.1456	0.0000
	7	0.0656	10	0.0579	1.1330	0.2865	11.8204	0.0000
	8	0.007	10	0.0449	0.1559	0.8796	16.5479	0.0000
	9	-0.064	10	0.0366	1.7486	0.1143	22.2404	0.0000
	10	0.0019	10	0.0504	0.0377	0.9708	14.8433	0.0000
Nunhead Cemetery	11	0.0134	10	0.0271	0.4945	0.6328	27.1808	0.0000
	12	-0.0197	10	0.031	0.6355	0.5409	24.8290	0.0000
	13	0.0737	10	0.1116	0.6604	0.5255	6.0600	0.0002
	14	0.0034	10	0.0533	0.0638	0.9505	14.0075	0.0000
	15	0.0161	10	0.0351	0.4587	0.6573	20.9088	0.0000
	16	-0.0462	10	0.077	0.6000	0.5633	10.3403	0.0000
	17	-0.0152	10	0.0401	0.3791	0.7134	19.0823	0.0000
	18	0.0415	10	0.0727	0.5708	0.5821	9.7455	0.0000
	19	0.0079	10	0.0231	0.3420	0.7402	32.1255	0.0000
	20	0.0135	10	0.0423	0.3191	0.7569	17.4113	0.0000
Regent's Park	21	0.0764	10	0.0608	1.2566	0.2405	11.0789	0.0000
	22	0.0256	10	0.0644	0.3975	0.7003	11.2484	0.0000
	23	0.0208	10	0.0433	0.4804	0.6424	16.8406	0.0000
	24	-0.0151	10	0.035	0.4314	0.6763	21.8600	0.0000
	25	0.0028	10	0.0351	0.0798	0.9382	21.2877	0.0000
	26	0.0657	10	0.0657	1.0000	0.3434	10.4155	0.0000
	27	0.0191	10	0.0529	0.3611	0.7264	13.8166	0.0000
	28	0.1473	10	0.0603	2.4428	0.0372	9.9950	0.0000
	29	-0.0002	10	0.0508	0.0039	0.9969	14.7677	0.0000
	30	-0.0405	10	0.0367	1.1035	0.2984	21.5395	0.0000
Millennium Village	31	-0.0411	10	0.0119	3.4538	0.0072	66.4790	0.0000
	32	-0.0596	10	0.0402	1.4826	0.1723	20.1393	0.0000
	33	0.0572	10	0.0768	0.7448	0.4754	9.0208	0.0000
	34	0.0426	10	0.0634	0.6719	0.5185	11.1577	0.0000
	35	-0.068	8	0.0711	0.9564	0.3639	11.5049	0.0000
	36	0.0133	10	0.0461	0.2885	0.7795	15.9805	0.0000
	37	-0.0158	10	0.035	0.4514	0.6624	21.8800	0.0000
	38	-0.0089	10	0.0258	0.3450	0.738	29.4147	0.0000
	39	-0.0666	10	0.0381	1.7480	0.1144	21.4331	0.0000
	40	0.0265	10	0.0289	0.9170	0.3831	25.0346	0.0000

Table 6. Estimated number of colonies per *Bombus* sampling site. When calculating the average number of *B. terrestris* colonies visiting sites, the Regent's Park site was excluded in order to equalize the sample sizes (98-100 workers) of included sites.

[1] Denotes estimates without correction for unsampled colonies.

Site	*B. terrestris* No. of workers	*B. terrestris* No. of colonies [1]	*B. pascuorum* No. of workers	*B. pascuorum* No. of colonies [1]
Nunhead Cemetery	100	58	100	55
Barnes Common	99	66	100	60
Regent's Park	74	30	100	53
Millennium Village	100	61	98	40
Thames Barrier	98	69	-	-
Mean	99.2	63.5	99.5	52

Table 7a. Multilocus pairwise F_{st} values between pairs of sample sites for *B. terrestris*. Estimated values of F_{st} were tested for significance by randomly assigning genotypes to sites. There were no significant pairwise F_{st} values after Bonferroni correction for multiple tests.

Site	Nunhead Cemetery	Barnes Common	Regent's Park	Millennium Village	Thames Barrier	Tolworth	Hanwell Cemetery	Woodgrange Park	St. James' Lane	Beddington	Grove Park
Nunhead Cemetery	0										
Barnes Common	-0.0122	0									
Regent's Park	0.0018	0.0011	0								
Millennium Village	-0.0237	-0.0118	0.0134	0							
Thames Barrier	-0.0003	0.0043	0.0285	0.0163	0						
Tolworth	0.0099	0.0155	0.014	0.0074	0.047	0					
Hanwell Cemetery	-0.0275	-0.0076	-0.0053	-0.0098	-0.011	0.0031	0				
Woodgrange Park	-0.0191	-0.0072	-0.0046	-0.0058	0.0061	0.0041	-0.0303	0			
St. James' Lane	0.0237	0.0153	0.0117	0.0051	0.0457	0.023	0.0136	0.0275	0		
Beddington	-0.0327	-0.0092	-0.0028	0.0001	-0.0093	0.0013	-0.026	-0.0048	0.0177	0	
Grove Park	-0.0206	0.0015	0.0053	-0.0069	-0.0052	0.0244	-0.0202	-0.0149	0.023	-0.0116	0

Table 7b. Multilocus pairwise F_{st} values between pairs of sample sites for *B. pascuorum*. Estimated values of F_{st} were tested for significance by randomly assigning genotypes to sites. Significant values, after Bonferroni correction for multiple tests ($p < 0.05$), are indicated with an asterisk.

Site	Barnes Common	Nunhead Cemetery	Regent's Park	Millennium Village	Woodgrange Park	Beddington	Grove Park	Hanwell Cemetery	Tolworth	St. James' Lane
Barnes Common	0									
Nunhead Cemetery	-0.0048	0								
Regent's Park	0.0183	0.0265	0							
Millennium Village	0.006	0.0291	0.034	0						
Woodgrange Park	-0.0044	0.0055	0.0339	0.0076	0					
Beddington	-0.0163	-0.0028	0.0113	-0.0064	-0.0023	0				
Grove Park	-0.0015	-0/0021	0.0367	0.0161	0.005	0.0031	0			
Hanwell Cemetery	0.0052	0.0161	*0.0385	0.0244	-0.0112	0.0044	0.0238	0		
Tolworth	-0.0073	0.0275	0.0262	0.0093	-0.0015	-0.0259	0.0262	-0.0023	0	
St. James' Lane	-0.0029	0.0139	0.0283	0.0114	-0.0019	-0.018	0.0055	0.0151	-0.017	0

Table 8. Time in minutes taken to collect 10 workers at each patch at each site. Note that where n<100 not all workers had 10 workers collected from them.

Species	Site	Overall n workers	Time taken to collect 10 bees per patch (min)										
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**
B. terrestris	Nunhead Cemetery	100	56	49	24	65	34	29	33	10	48	61	41
B. terrestris	Barnes Common	99	8	21	29	54	33	165	30	35	35	30	44
B. terrestris	Regent's Park	74	27	14	17	27	19	31	29	31	10	14	22
B. terrestris	Millennium Village	100	65	47	41	55	21	46	43	80	31	33	46
B. terrestris	Thames Barrier	98	54	43	14	60	36	70	110	81	40	29	54
B. pascuorum	Nunhead Cemetery	100	17	27	32	61	49	43	16	59	74	66	44
B. pascuorum	Barnes Common	100	35	25	15	20	9	10	11	12	15	16	17
B. pascuorum	Regent's Park	100	69	7	30	31	21	25	14	29	27	22	27
B. pascuorum	Millennium Village	98	46	25	20	15	27	57	22	72	50	40	37

Figure 1. A map to show the distribution of the genetic sampling sites. Up to 100 workers of each species of *B. terrestris* and *B. pascuorum* were collected from the 'patch use' sites. Ten workers of each species were collected from each of the 'population sample' sites.

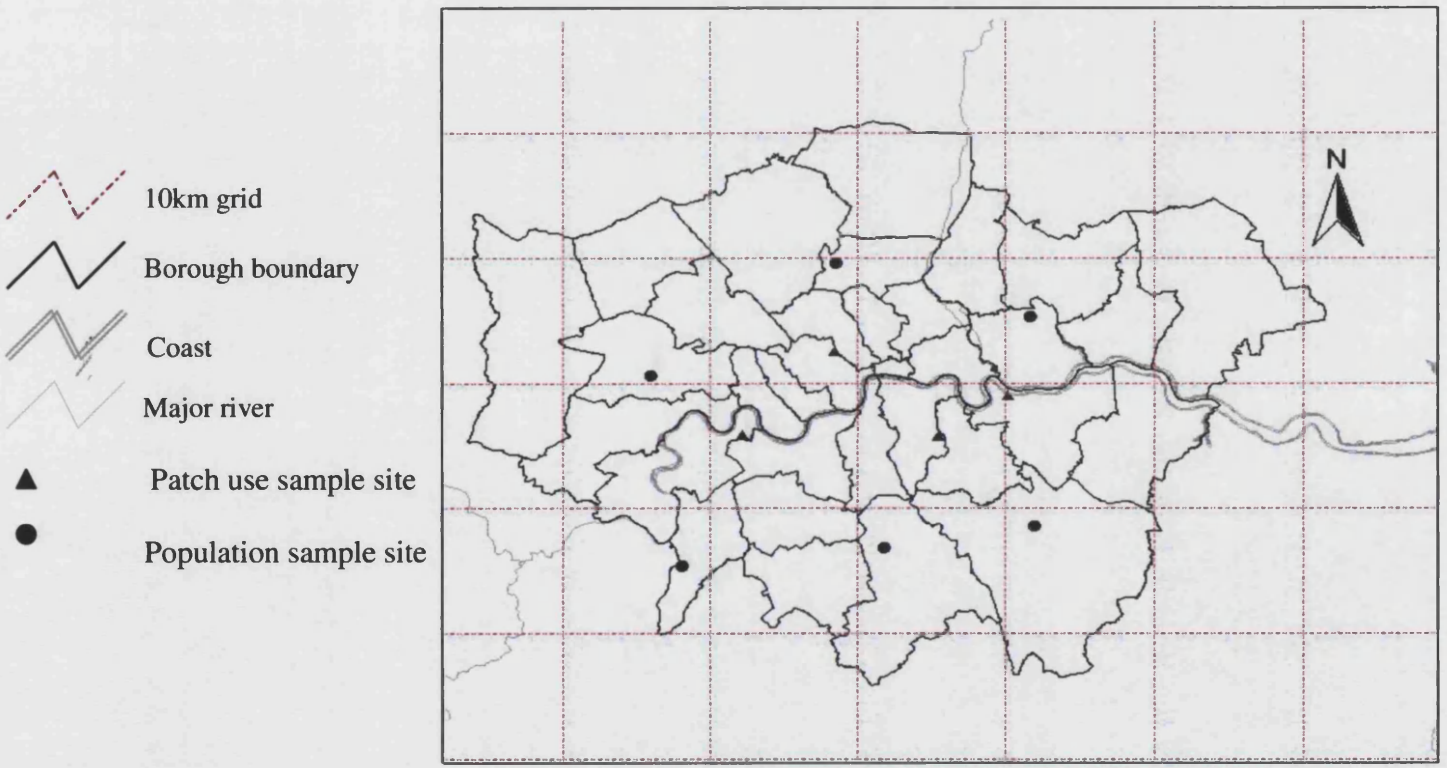
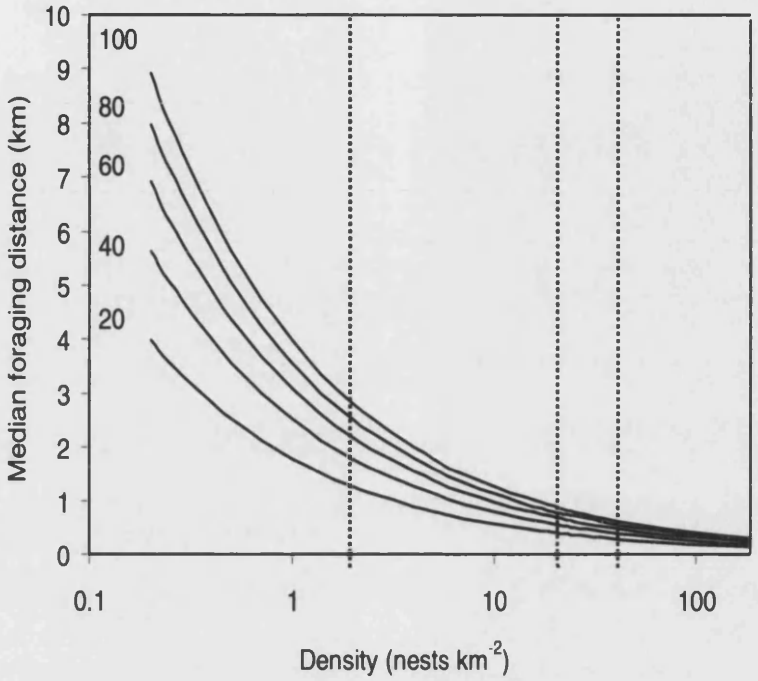


Figure 2. Estimated median foraging distances as a function of nest density for workers visiting a site attracting K = 20-100 colonies; dotted vertical lines show nest densities of 2, 20 and 40 nests per km^2.



Chapter 6

Discussion

The overall aim of this Ph.D. study was to generate information, through scientific study, of use to biologists in the conservation of bumble bees, especially those species and populations occurring in urban areas. The main scientific conclusions are outlined below, followed by the conservation recommendations arising from them.

Scientific conclusions

In the survey of five urban habitat types spread over thirty sites in London, I identified six 'mainland ubiquitous' bumble bee species and one Biodiversity Action Plan species (BAP), *B. humilis* (Chapter 2). A second BAP species, *B. sylvarum*, and an English Nature's Species Recovery Programme species, *B. ruderarius*, were later identified from a focussed study, but, due to their paucity, very little can be concluded from records of these species (Chapter 3). Five of the 'mainland ubiquitous' species were found to be common and widespread, but *B. hortorum* was much less frequently encountered and had a limited distribution across the city and habitats. The greatest abundance of foraging workers was recorded in south London and the greatest diversity of bees in the east. Of all of the habitats, wasteland and gardens supported the greatest abundance of workers; each of these supported six of the seven *Bombus* species. There was an equal abundance in the availability of native and introduced flowers, but there were significantly more native flowers in cemeteries, wastelands and common land. *B. pascuorum* and *B. pratorum* were the only species of bumble bee to visit native species and perennial flowers more frequently. *B. lapidarius* visited annual flowers more frequently, *B. terrestris / B. lucorum* visited biennials more frequently and *B. hortorum* and *B. humilis* visited annuals, biennials, perennials and shrubs equally. The common wildflowers *Trifolium repens* and *Rubus fruticosus*

were favourite forage plants of *B. terrestris* / *B. lucorum*, *B. pratorum* and *B. lapidarius*. *B. pascuorum* was found to prefer forage from the *Fabaceae* and *Lamiaceae* families (Chapter 2). In Chapter 3, twelve sites in east London were identified as supporting *B. humilis* throughout the flying season. The distribution of this species was found to follow the strip of derelict Thames-side industrial land on the eastern side of London as far west as the Millennium Dome, and the River Lea as far north as the Walthamstow Marshes. The phenology of *B. humilis* in London was established. The number of *B. humilis* queens peaked in mid-June and the number of workers in late-July. The garden habitat of the Thames Barrier Park was found to support the greatest number of foraging *B. humilis*. Out of the seven species of *Bombus* identified at this site, *B. humilis* was the fourth most abundant species and made up 19% of all bumble bees observed. In this study, species of plant native to the U.K. were not necessarily favoured forage of *B. humilis*, which was found to favour flowers in the Lamiaceae family, particularly the garden variety *Lavandula* x *intermedia* 'Dutch'. The greatest potential competitor of *B. humilis* was found to be *B. pascuorum* (Chapter 4). The presence of workers of other species, including *B. pascuorum*, has been shown to reduce the time spent by *B. humilis* workers foraging at patches of flowers. In the absence of all competitors the length of time that *B. humilis* workers spend foraging at patches of flowers increases. The removal of *B. humilis*, however, had no effect on the foraging duration of workers of other species. However, due to methodological constraints, the results have alternative explanations. Further work needs to be carried out, with more rigorous experiments to clarify the exact nature of the relationship of *B. humilis* and its competitors during foraging. If the foraging behaviour of *B. humilis* is affected by other species this may in part why

B. humilis is found more frequently in the very best flower-rich habitats whereas *B. pascuorum* can also be found in poorer quality habitats.

Aspects of the foraging ecology of the two most common species of bumble bee (*B. terrestris* and *B. pascuorum*) have been explored at different spatial scales using microsatellite analysis (Chapter 5). Neither *B. terrestris* nor *B. pascuorum* workers were found to forage preferentially with their nest-mates on patches of flowers. The estimated number of *B. terrestris* colonies foraging at a site was consistently higher than the number of *B. pascuorum* colonies. The mean minimum number of *B. terrestris* colonies identified per site was 63 and the mean minimum number of *B. pascuorum* colonies identified per site was 52. Overall the greatest number of colonies were identified at the Thames Barrier Park, with an estimated 69 colonies of *B. terrestris* identified foraging over an area of 0.88ha. No inbreeding effects were identified and no genetic differentiation could be detected in either species across the study sites throughout London (Chapter 5).

This thesis has shown that bumble bees can thrive in the urban environment, which therefore has the potential to provide a refuge for species of bumble bee declining in the surrounding countryside. It is an open issue whether these findings can be applied successfully to other urban environments in Britain or even Europe. There are aspects of London that make it a typical city, for example high levels of pollution and overcrowding. However, London's enormous size is atypical of most cities, and geographical location is unique; it is also renowned for its open spaces and terraced

housing with gardens, so in that respect it may not be typical of all urban areas. Nevertheless, the scientific conclusions can, in principal be applicable universally.

Conservation recommendations

The presence of six widespread species (Chapter 2) and three rare species of British *Bombus* (Chapter 3) indicates that the flower-rich green spaces of London may be providing, or have the potential to provide, a refuge for bumble bees that suffer from a lack of forage and nest-sites in the surrounding countryside. This thesis has shown that wastelands and gardens are especially important as bumble bee habitats, since they support the greatest diversity of species and abundance of workers (Chapter 2). However, the fact that no single habitat was found to support all seven species of bumble bee highlights the importance of the diversity of habitats that is provided by urban areas. It also shows that the conservation of bumble bees does not simply involve preserving a few examples of one habitat type. One of the biggest surprises of the London-wide species survey was the paucity of *B. hortorum* sightings (Chapter 2). I found that gardens were, as expected, the preferred habitat of *B. hortorum*, and it would be reasonable to expect this species to be common in an urban area with so much of its preferred habitat, or to be at least nearly as abundant as the other common species. Even after taking into account the small colony size of this species, I did not find this to be the case in this study. *B. hortorum* provides an interesting opportunity to carry out 'damage limitation' to halt the decline of Britain's most widespread and longest-tongued bumble bees species (Prys-Jones & Corbet, 1991). Corbet (2000) argues that limited resources would be better utilised trying to conserve *B. hortorum* rather than, for example, a poorly understood species such as *B. sylvarum*. Since *B.*

hortorum is the longest-tongued British bumble bee species by far, it is very likely that there are some species of plant that rely very heavily on it for pollination. As one of the more common and widespread species, there are sufficient data on the foraging and habitat usage of *B. hortorum* (e.g. Comba *et al.*, 1999b; Corbet *et al.*, 2001; Fussell & Corbet, 1993) to initiate a nationwide promotion of the planting of its favourite forage species such as *Trifolium pratense* (red clover), *Digitalis purpurea* (foxglove) and *Salvia pratensis* (meadow clary). Gardening is currently enjoying a popular renaissance and the so the possibility exists to convey a substantial body of information to gardeners through newspapers and television. The promotion of *B. hortorum*-friendly planting through television programmes, garden centres and wildlife trusts will not only improve gardens for *B. hortorum* but also raise the conservation profile of the genus and of other pollinators.

Two of the conservation management recommendations arising from this study are the recommendations to conduct forage habitat enrichment in the form of sowing wildflower seed mixtures to enhance common land and to improve public parks for bumble bees through the informed selection of garden plant varieties (Comba *et al.*, 1999a, 1999b; Corbet *et al.*, 2001). This is especially important because, as wastelands are developed and lost from the urban habitat matrix, their forage potential needs to be replaced. This might be partially achieved by enhancing existing habitats such as public parks and cemeteries, which currently under-perform as bumble bee habitat due to their low diversity and abundance of forage (Chapter 2). The restoration of habitat for bumble bees and other pollinators using wildflower seed mixtures is not a new concept in the agricultural environment and is positively encouraged under the

Countryside Stewardship Scheme (MAFF, 1998) and the Arable Stewardship Pilot Scheme (MAFF, 1999). A mixture specifically composed for bumble bees is currently under development (Edwards, 2002b) and early versions are being deployed in experimental trials. Although the planting of wildflower meadow areas in the urban environment is primarily for 'wildlife', the aesthetics of the meadow is clearly important for a public recreational area. For this reason the application of an agricultural seedmix in an urban environment may not be appropriate. For example, one of the criticisms made of the wildflower meadow at the Thames Barrier Park by the Park gardeners (personal communication) is that a great deal of labour intensive work has to be put into it to prevent the area becoming dominated by species such as thistles and ragwort, which are attractive as wildflowers, but only in low densities. The main reason that the meadow was mown before the end of flowering and before *B. humilis* had completed its cycle (Chapter 3) was to prevent the more competitive flower species from going to seed, thereby boosting their numbers the following year. The wildflower seed-mix industry is growing and mixes are constantly being developed so they should never be excluded as an option for bumble bee conservation. Further work is needed so that the requirements of both gardeners and pollinators are catered for. In the development of large-scale garden schemes, the importance of using plant species and varieties specially selected for the purpose cannot be overstated. Some work has been carried out to assess the value of different varieties of species of garden flowers to pollinators (Comba *et al.*, 1999a, 1999b; Corbet *et al.*, 2001). For example, *Lotus corniculatus*, a species that should be planted to encourage *B. humilis,* comes as a variant with a single layer of petals, which attracts many foragers, whereas the double-layered variant produces no nectar and is

of no value to pollinators (Corbet *et al.*, 2001). Clearly, formally planted parks have tremendous potential for pollinators but only if they are planted appropriately. The contents of private gardens are usually decided by one or a few householders, which leads to an incredible diversity of plants across private gardens collectively. This diversity is generally only limited by what is available at gardens centres; however, the contents of formal parks tend to be more centrally controlled, resulting in many parks exhibiting the same varieties of plants from the same source. Further work needs to be conducted along the lines of that of Corbet (2001) and the people in charge of the planting schemes for public areas need to be made aware of the impact of their choices.

This thesis has shown that, although *B. humilis* can be found foraging on wasteland sites in London, it is not as strongly associated with this habitat in the urban environment as was once thought (London Biodiversity Partnership, 2001a). Instead, *B. humilis* has been shown to exploit a wide range of forage species, both native wildflowers and common garden species (Chapter 3). This is very encouraging from a conservation point of view because it allows some flexibility in conservation management in the face of the inevitable development of wastelands. Obviously the creation of nature reserves should not be discouraged, but with the reported need for 366,000 extra homes in London by 2016 (Mayor of London, 2004), the creation of protected areas for wildlife will not necessarily be a priority. Rather than fruitlessly resisting development and watching the habitat matrix disintegrate, conservationists should aim to work together with developers to incorporate genuinely pollinator-friendly elements into the landscaping that accompanies development. The recently

completed Thames Barrier Park and Millennium Village in London's East End provide excellent examples of how this can be achieved, with the reduction in the physical area of bumble bee habitat being compensated for by improved quality of the habitat in the form of an increased density and diversity of flowers. Not only did the Thames Barrier Park support the greatest numbers of *B. humilis* and *B. sylvarum* (Chapter 3), but also its wildflower meadow drew foraging *B. terrestris* workers from more colonies (Chapter 5) than any other site sampled. The development of both sites has included substantial public green space planted with a combination of formal gardens and wildflower seed mix, which the present thesis showed to provide excellent forage. Both sites also provide a lawn rich with red and white clover and patches of *Lotus corniculatus*, which are important sources of nectar and pollen for *B. humilis* (Chapters 3 and 4) (Goulson & Darvill, 2004). The Millennium Village goes a step further and its construction has actually involved the creation of a wetland nature reserve; this reserve included plant species such as yellow flag (*Iris pseudacorus*), which proved a popular source of pollen for foraging *B. humilis* queens (Chapter 3). The data on the phenology of *B. humilis* collected in this thesis have already been used to advise garden managers on mowing regimes that will minimise the mechanical destruction of nests before colonies have completed their colony-cycle. This thesis has not directly examined nesting requirements of any species in London. To provide more complete information on how to enhance the urban environment for bumble bees, further work on nesting requirements needs to be carried out for all species, particularly *B. humilis*.

Although British bumble bees are undergoing severe declines, they tend not to receive as much attention as declining vertebrate species. One potential cause of this is lack of information stemming from the fact that the population biology of bumble bees is difficult to study. The development of a genetic technique for the analysis of spatial foraging patterns and resource sharing has removed a significant barrier to such work (Chapter 5). In two of the most common British species (*B. terrestris* and *B. pascuorum*), the number of colonies of bumble bees utilising an area for forage has been estimated for the first time and baseline data for further studies of population genetics have been provided. The fact that so many colonies have been identified foraging at sites does indicate that, in the case of *B. terrestris* and *B. pascuorum* at least, an urban environment seems to support healthy populations. The fact that patches of forage are attracting large numbers of colonies to them, potentially over distances of several kilometres, has two main conservation management implications, for common and rare species alike. First, it suggests that bumble bees are not nesting and foraging at the same sites (since nests are unlikely to occur in as high a density at the study sites as the densities of colonies foraging at the sites that I recorded). These habitats therefore need to be considered as separate units when it comes to management strategies. Second, it means that any conservation strategies should be planned and executed on an appropriate scale. For example, rather than just one or two boroughs being pro-active, there needs to be a city-wide effort to maintain and even enhance the matrix of patches of forage and nesting habitat that already exist. The genetic technique described in Chapter 5 (this thesis) (Chapman *et al.*, 2003), combined with non-lethal sampling of DNA (Holehouse *et al.*, 2003), can now be

used to improve our knowledge of the behaviour and population dynamics of rare species for conservation purposes. Studies of this nature should be made a priority.

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Appendix A

Environmental variables. The description of the weather was made by sight on arrival at the study site. A thermometer was placed at the start of the transect on arrival and the temperature read on departure.

Study site	Date	Weather	Temperature (°C)
Brompton Cemetery	03/06/2000	Sunny	21
Queensmill Road	03/06/2000	Sunny	23
St. Luke's Church	03/06/2000	Sunny	24
Highgate Cemetery	05/06/2000	Overcast	16
Sand's End	06/06/2000	Sunny	17
Walpole Park	06/06/2000	Overcast	16
Prince's Gardens	06/06/2000	Sunny	18
Regent's Park	07/06/2000	Sunny	21
St. James Lane	10/06/2000	Sunny	19
Hampstead Heath	12/06/2000	Sunny	24
Barnes Common	15/06/2000	Overcast	17
Berrylands	15/06/2000	Overcast	19
St. Andrew's Square	15/06/2000	Overcast	19
Morden	26/06/2000	Sunny	16
Tolworth	26/06/2000	Sunny	18
Cannon Hill Common	26/06/2000	Sunny	17
Nunhead	27/06/2000	Overcast	20
Brooks Avenue	27/06/2000	Overcast	22
Sexby Gardens	28/06/2000	Sunny	18
Ossary Road	28/06/2000	Sunny	19
Burgess Park	29/06/2000	Cloudy	18
Pymer's Mead	29/06/2000	Cloudy	18
Tollington Road	03/07/2000	Sunny	19
Woodgrange Park	03/07/2000	Sunny	24
Ferndale Street	06/07/2000	Sunny	20
Wanstead Flats	07/07/2000	Overcast	16
West Ham Park	10/07/2000	Overcast	15
Jenner Avenue	12/07/2000	Overcast	16
Sand's End	12/07/2000	Overcast	18
Prince's Gardens	13/07/2000	Overcast	18
Ealing Common	14/07/2000	Overcast	17
Hanwell Cemetery	14/07/2000	Overcast	18
Brompton Cemetery	16/07/2000	Cloudy	17
St. Luke's Church	16/07/2000	Cloudy	16
Queensmill Road	16/07/2000	Cloudy	16
Highgate Cemetery	17/07/2000	Sunny	18
Hampstead Heath	17/07/2000	Sunny	21
Regent's Park	18/07/2000	Sunny	21
St. James Lane	18/07/2000	Sunny	24
Barnes Common	20/07/2000	Sunny	21
St. Andrew's Square	20/07/2000	Sunny	23
Berrylands	20/07/2000	Sunny	24
Ossary Road	21/07/2000	Cloudy	24

Environmental variables continued.

Study site	Date	Weather	Temperature (°C)
Sexby Gardens	21/07/2000	Cloudy	24
Burgess Park	22/07/2000	Overcast	16
Pymer's Mead	22/07/2000	Overcast	17
Brooks Avenue	22/07/2000	Cloudy	20
Nunhead Cemetery	24/07/2000	Overcast	15
Tollington Road	24/07/2000	Overcast	16
Morden Cemetery	25/07/2000	Overcast	16
Cannon Hill Common	25/07/2000	Overcast	16
Tolworth	25/07/2000	Sunny	18
Ferndale Street.	26/07/2000	Sunny	22
West Ham Park	26/07/2000	Sunny	22
Woodgrange Park	26/07/2000	Sunny	19
Wanstead Flats	26/07/2000	Sunny	22
Jenner Avenue	27/07/2000	Sunny	21
Walpole Park	27/07/2000	Sunny	21
Hanwell Cemetery	27/07/2000	Overcast	22
Ealing Common	27/07/2000	Overcast	22
St. Luke's Church	01/08/2000	Sunny	20
Morden Cemetery	10/08/2000	Cloudy	21
Cannon Hill Common	10/08/2000	Cloudy	23
Sand's End	13/08/2000	Cloudy	19
Queensmill Road	13/08/2000	Cloudy	22
Nunhead	15/08/2000	Sunny	20
Sexby Gardens	15/08/2000	Sunny	23
Burgess Park	15/08/2000	Sunny	22
Berrylands	18/08/2000	Cloudy	19
St. Andrew's Square	18/08/2000	Cloudy	20
Regent's Park	18/08/2000	Cloudy	20
Walpole Park	19/08/2000	Sunny	19
Hanwell Cemetery	19/08/2000	Sunny	20
Ealing Common	19/08/2000	Sunny	22
Ossary Road	21/08/2000	Sunny	18
Pymer's Mead	21/08/2000	Sunny	20
Wanstead Flats	22/08/2000	Sunny	20
West Ham Park	22/08/2000	Sunny	23
Brooks Avenue	22/08/2000	Sunny	21
St. James Lane	23/08/2000	Sunny	22

Appendix B

The list of flower species recorded during forage availability survey. The habitats that each species was observed in and the total number of squares that each species was recorded in shown below. To sample the availability of potential forage I placed a $1m^2$ quadrat at 15m intervals along the 100m transect. The quadrat was sub-divided with string into one hundred $10cm^2$ units, known as squares from here on. Each square was surveyed for the presence or absence of inflorescences. Where inflorescences were present in a square they were identified to species and their presence was mapped by square onto a standardised data sheet. Using this method all of the plants in flower at the time, within the quadrat, were identified to species and the area they covered within the quadrat calculated. Whether a species is native to the U.K. or not and whether it is classified as annual (A), biennial, (B), Perennial (P) or a shrub (S) is shown.

Scientific name	Family	Native	A,B P,S	Cemetery	Common	Garden	Park	Wasteland	
				Number of squares recorded in					Total
Acanthus spinosus	Acanthaceae	No	P				45		45
Achillea millefolium	Asteraceae	Yes	P	6	39			40	85
Ageratum houstoniatum	Asteraceae	No	A				48		48
Agrimonia eupatoria	Rosaceae	Yes	P	43	5				48
Alcea rosa	Malvaceae	No	B			32			32
Alchemilla mollis	Rosaceae	No	P	12	6	22			40
Alchemilla vulgaris	Rosaceae	Yes	P	14	5				19
Alliaria petiolata	Brassicaceae	Yes	B					54	54
Alstroemeria	Liliaceae	No	P			60			60
Anagallis arvensis	Primulaceae	Yes	A	5					5
Anemone huphensis	Ranunculaceae	No	P			50			50
Anthriscus sylvestris	Apiaceae	Yes	P				73	47	120
Antirrhinum majus	Scrophulariaceae	No	P					2	2
Aquilegia vulgaris	Ranunculaceae	Yes	P				2		2
Arctium lappa	Asteraceae	Yes	B					118	118
Arenaria serphyllifolia	Caryophyllaceae	Yes	B	8					8
Armoracia rusticana	Brassicaceae	No	P					30	30
Artemesia arbrotanum	Asteraceae	No	P				27	7	34
Artemesia schmidtiana	Asteraceae	No	P				17		17
Artemesia vulgaris	Asteraceae	Yes	P		4			61	65

Aster novi-belgii	Asteraceae	No	P			84			84
Aster x salignus	Asteraceae	No	P				5	15	20
Ballota nigra	Lamiaceae	Yes	P	217			0	154	371
Bellis perennis	Asteraceae	Yes	P	5		12		11	28
Bracteantha bracteata	Bracteantha	No	A			18			18
Brassica napus	Brassicaceae	No	B					262	262
Buddleja davidii	Buddlejaceae	No	S					229	229
Calystegia silvatica	Convolvulaceae	No	P	16			31	94	141
Cardaria draba	Brassicaceae	No	P		12				12
Carduus crispus	Asteraceae	Yes	A		20	0			20
Carduus tenuflorus	Asteraceae	Yes	A		9			27	36
Ceanothus impressus	Rhamnaceae	No	S			50			50
Centaurea montana	Asteraceae	No	P	20					20
Centaurea nigra	Asteraceae	Yes	P	38	96			3	137
Centaurea scabiosa	Asteraceae	Yes	P	5					5
Ceratostigma wilmotianum	Plumbaginacea	No	S			64			64
Cerinthe major 'purpurascens'	Boraginaceae	No	P			12			12
Chenopodium bonus-henricus	Chenopodiaceae	Yes	P					2	2
Cirsium arvense	Asteraceae	Yes	P		83			165	248
Cirsium vulgare	Asteraceae	Yes	B	3	52	0		3	58
Clematis montana	Ranunculaceae	No	P			200			200
Convolvulus arvensis	Convolvulaceae	Yes	P		44			10	54
Crepis capillaris	Asteraceae	Yes	B	11	3			5	19
Crepis vesicaria	Asteraceae	No	P		8				8
Crocosima 'Lucifer'	Iridaceae	No	P			31			31
Cytisus scoparius	Fabaceae	Yes	S			97	120	35	252
Dahlia	Asteraceae	No	P			300			300
Delphinium 'Blue Nile'	Ranunculaceae	No	P			4			4
Deutzia	Hydrangeaceae	No	S			80			80
Digitalis purpurea	Scrophulariaceae	Yes	B	2			3		5
Dipsacus fullonum	Dipsacaceae	Yes	B	45				33	78
Epilobium hirsutum	Onagraceae	Yes	P				20	16	36
Epilobium montanum	Onagraceae	Yes	P	6			8		14
Foeniculum vulgare	Apiaceae	No	P					24	24
Galega officinalis	Fabaceae	No	P					92	92
Gentiana asclepiadea	Gentianaceae	No	P				28		28
Geranium dissectum	Geraniaceae	Yes	A	12	2	0	0		14
Geranium endressii	Geraniaceae	No	P	19			215		234
Geranium himalayense	Geraniaceae	No	P			0	104		104
Geranium palustre	Geraniaceae	Yes	P	51					51
Geranium phaeum	Geraniaceae	No	P				100		100
Geranium pratense	Geraniaceae	Yes	P			48	62		110
Geranium pyrenaicum	Geraniaceae	Yes	P		154				154
Geranium renardii	Geraniaceae	No	P			40	90		130
Geranium robertianum	Geraniaceae	Yes	A	12					12
Geranium x Oxonianium	Geraniaceae	No	P			16			16
Geum urbanum	Rosaceae	Yes	P				2		2
Hebe 'Bowles' variety	Scrophulariaceae	No	S			107			107
Hebe takaiensis	Scrophulariaceae	No	S			54			54

Hemerocallis	Liliaceae	No	P				172		172
Hieracium umbellatum	Asteraceae	Yes	P		10				10
Hieracium vulgatum	Asteraceae	Yes	P		14				14
Hydrangea macrophylla	Hydrangeaceae	No	S			319			319
Hydrangea villosa	Hydrangeaceae	No	S			200			200
Hypericum 'Hidecote'	Clusiaceae	No	S				204		204
Hypericum inodorum	Clusiaceae	No	S				200		200
Hypericum perforatum	Clusiaceae	Yes	P	9					9
Hypochaeris glabra	Asteraceae	Yes	P		16				16
Hypochaeris radicata	Asteraceae	Yes	P	51	49				100
Impatiens glandulifera	Balsaminaceae	No	A			98			98
Iris pseudacorus	Iridaceae	Yes	P			30	39		69
Isatis tinctoria	Brassicaceae	No	P					32	32
Lactuca serriola	Asteraceae	Yes	B					4	4
Lactuca virosa	Asteraceae	Yes	B		2			7	9
Lamium album	Lamiaceae	Yes	P	6		31	37	46	120
Lamiastrum galeobdolon	Lamiaceae	Yes	P				100		100
Lamium maculatum	Lamiaceae	No	P			70			70
Lamium purpureum	Lamiaceae	Yes	A					9	9
Lapsana communis	Asteraceae	Yes	A				4	1	5
Lathyrus latifolius	Fabaceae	No	P	29					29
Lathyrus odoratus	Fabaceae	No	A			49			49
Lathyrus pratensis	Fabaceae	Yes	P	0					0
Lavatera 'Rosea'	Malvaceae	No	S			304			304
Lavandula angustifolia	Lamiaceae	No	S			40	39		79
Lavandula angustifolia 'Hidecote'	Lamiaceae	No	S			50			50
Lavandula stoechas	Lamiaceae	No	S			160			160
Leontodon saxatalis	Asteraceae	Yes	P	7	39				46
Leucanthemum vulgare	Asteraceae	Yes	P	23				37	60
Ligustrum ovalifolium	Oleaceae	No	S				200		200
Linaria vulgaris	Scrophulariaceae	Yes	P					2	2
Lonicera periclymenum	Caprifoliaceae	Yes	P	58			69		127
Lotus corniculatus	Fabaceae	Yes	P	64	41				105
Lotus pendunculus	Fabaceae	Yes	P	11				0	11
Lychnis flos-cuculi	Caryophyllaceae	Yes	P	9					9
Lysimachia nummularia	Primulaceae	Yes	P	12					12
Lythrum salicaria	Lythraceae	Yes	P			100			100
Malva moschata	Malvaceae	Yes	P					9	9
Malva sylvestris	Malvaceae	Yes	P					76	76
Medicago lupulina	Fabaceae	Yes	A	8	33			252	293
Medicago sativa	Fabaceae	No	P					15	15
Melilotus albus	Fabaceae	No	A					202	202
Melilotus officinalis	Fabaceae	No	B					62	62
Mentha spicata	Lamiaceae	No	P					21	21
Mysotis sylvatica	Boraginaceae	Yes	P			30			30
Nepeta cataria	Lamiaceae	Yes	P			38			38
Oenothera glazoviana	Onagraceae	No	A	1				1	2
Papaver orientale	Papaveraceae	No	A			20			20
Papaver rhoeas	Papaveraceae	Yes	A					22	22
Pelargonium	Geraniaceae	No	P			200	472	0	672
Penstemon	Scrophulariaceae	No	P			200			200

Pentaglottis sempervirens	Boraginaceae	No	P				6		6
Persicaria maculosa	Polygonaceae	Yes	A	4				0	4
Picris echioides	Asteraceae	No	A	2				298	300
Picris hieracoides	Asteraceae	Yes	P		36			27	63
Plantago lanceolata	Plantaginaceae	Yes	P	4	143			42	189
Polygonium avicularia	Polygonaceae	Yes	P					17	17
Potentilla fruticosa	Rosaceae	Yes	S			530			530
Potentilla reptans	Rosaceae	Yes	P	4					4
Prunella vulgaris	Lamiaceae	Yes	P	74	3			1	78
Pulicaria dysenterica	Asteraceae	Yes	P					4	4
Ranunculus acris	Asteraceae	Yes	P	7	45		3		55
Ranunculus auricormus	Asteraceae	Yes	P	17	8				25
Ranunculus repens	Asteraceae	Yes	P	14	5				19
Reseda lutea	Resedaceae	Yes	P					20	20
Rosa 'Abraham Darby'	Rosaceae	No	S			57	94		151
Rosa canina	Rosaceae	Yes	S					100	100
Rosa 'Iceberg'	Rosaceae	No	S			32	93		125
Rosmarinus officinalis	Lamiaceae	No	S			68			68
Rubus fruticosus	Rosaceae	Yes	S	471				78	549
Rudbekia hirta 'Marmalade'	Asteraceae	No	P			29			29
Rumex acetosella	Polygonaceae	Yes	P		10				10
Salvia farinacea	Lamiaceae	No	P			36	24		60
Salvia pratensis	Lamiaceae	Yes	P	3					3
Salvia sclaria 'turkestancia'	Lamiaceae	No	P			18			18
Sambucus nigra	Caprifoliaceae	Yes	S					42	42
Sedum rupestre	Crassulaceae	No	P	0			140		140
Senecio jacobaea	Asteraceae	Yes	P	21	69			229	319
Senecio squalidus	Asteraceae	No	P					27	27
Silene dioica	Caryophyllaceae	Yes	P	4					4
Silene latifolia	Caryophyllaceae	Yes	P					19	19
Sisymbrium officinale	Brassicaceae	Yes	B					24	24
Sisyrinchium striatum	Iridaceae	No	P	14		20			34
Solanum dulcamara	Solanaceae	Yes	P	12					12
Solidago canadensis	Asteraceae	No	P				40	183	223
Sonchus arvensis	Asteraceae	Yes	B					50	50
Spiraea japonica 'Little princess'	Rosaceae	No	S			56			56
Stellaria media	Caryophyllaceae	Yes	A	7					7
Symphoricarpus albus	Caprifoliaceae	No	S				300		300
Syringa vulgaris	Oleaceae	No	S			105			105
Tagetes 'Tangerine Dream'	Asteraceae	No	A			144	213		357
Taraxacum sp.	Asteraceae	Yes	P	3	20				23
Teucrium chamaedrys	Lamiaceae	Yes	S				80		80
Thymus 'herba barona'	Lamiaceae	No	S				140		140
Trifolium pratense	Fabaceae	Yes	P	64	47		5	40	156
Trifolium repens	Fabaceae	Yes	P	53	255	3	8	91	410
Tripleospermum inodoratum	Asteraceae	Yes	A					114	114
Verbascum nigrum	Scrophulariaceae	Yes	B	6					6
Veronica persica	Scrophulariaceae	No	A					73	73

Vicia tetrasperma	Fabaceae	Yes	A		11				11
Viola 'Jackanapes'	Violaceae	No	P			80			80
				1622	1398	4498	3682	3816	15016

Appendix C

Final genotypes used in analyses for *B. terrestris* and *B. pascuorum* nests. One *B. terrestris* nest sampled (N=8) was located in Brompton Cemetery, London (SW12). The second *B. terrestris* nest (N=6) and the *B. pascuorum* nest were located in a garden in Finchley, London (N3) where colonies are relocated to when they have had to be removed by the council's pest control unit.

Sample #	Nest #	Species	Collection Date	B10	B11	B96	B100	B124	B126
1	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/253	127/131
2	1	*B. pascuorum*	23/07/2001	174/174	135/135	215/225	138/138	249/253	127/127
3	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/253	127/127
4	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/221	138/138	249/253	127/131
5	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/257	127/127
6	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/253	127/127
7	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/253	127/131
8	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/253	127/127
9	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/253	127/127
10	1	*B. pascuorum*	23/07/2001	174/174	135/135	221/225	138/138	249/257	127/131
11	2	*B. terrestris*	23/07/2001	194/194	168/168	239/245	155/163	253/257	175/175
12	2	*B. terrestris*	23/07/2001	194/194	168/168	239/245	155/163	253/257	175/175
13	2	*B. terrestris*	23/07/2001	194/194	168/168	239/245	155/163	253/257	175/175
14	2	*B. terrestris*	23/07/2001	194/194	168/168	239/245	155/163	247/257	175/175
15	2	*B. terrestris*	23/07/2001	194/194	168/168	239/245	155/163	253/257	175/175
16	2	*B. terrestris*	23/07/2001	194/194	168/168	245/245	163/165	251/259	175/175
17	2	*B. terrestris*	23/07/2001	/	/	239/245	/	/	175/175
18	2	*B. terrestris*	23/07/2001	194/196	168/168	239/245	155/163	247/257	175/175
19	3	*B. terrestris*	21/06/2001	198/208	162/166	/	163/167	256/262	175/179
20	3	*B. terrestris*	21/06/2001	198/208	162/166	239/245	163/163	250/256	175/179
21	3	*B. terrestris*	21/06/2001	198/208	162/166	239/245	163/167	256/262	175/179
22	3	*B. terrestris*	21/06/2001	198/208	162/166	239/245	163/167	250/256	175/179
23	3	*B. terrestris*	21/06/2001	198/208	162/166	239/245	163/167	256/262	175/179
24	3	*B. terrestris*	21/06/2001	198/208	162/166	239/245	163/167	250/256	175/179

Appendix D

Final genotypes used in analyses for *B. terrestris*. A total of 531 workers were typed at an average of 5.8 (range 3-6) loci.

Sample ID	Sample Site	Patch	Date Collected	Locus B10	Locus B11	Locus B100	Locus B96	Locus B124	Locus B126
1	Nunhead Cemetery	1	26/06/2001	184/210	162/162	155/167	239/239	250/250	173/173
2	Nunhead Cemetery	1	26/06/2001	/	162/162	163/163	239/239	248/254	173/175
3	Nunhead Cemetery	1	26/06/2001	184/196	168/170	155/163	239/245	248/258	179/179
4	Nunhead Cemetery	1	26/06/2001	182/196	174/174	157/163	239/245	246/246	173/181
5	Nunhead Cemetery	1	26/06/2001	188/194	162/166	155/155	239/245	254/258	173/175
6	Nunhead Cemetery	1	26/06/2001	196/206	170/174	155/163	239/245	254/256	173/175
7	Nunhead Cemetery	1	26/06/2001	196/198	162/174	157/163	245/245	250/254	179/191
8	Nunhead Cemetery	1	26/06/2001	196/206	170/170	155/163	239/243	246/256	181/185
9	Nunhead Cemetery	1	26/06/2001	210/210	162/162	167/167	239/239	250/260	171/195
10	Nunhead Cemetery	1	26/06/2001	196/198	162/162	155/165	239/239	250/250	173/179
11	Nunhead Cemetery	2	26/06/2001	192/208	170/174	165/165	245/247	246/246	173/191
12	Nunhead Cemetery	2	26/06/2001	198/198	170/170	163/167	239/247	254/260	175/179
13	Nunhead Cemetery	2	26/06/2001	/	170/170	157/157	245/245	254/256	175/175
14	Nunhead Cemetery	2	26/06/2001	196/208	162/170	157/161	239/245	246/250	173/173
15	Nunhead Cemetery	2	26/06/2001	194/198	162/166	155/155	245/245	254/256	177/177
16	Nunhead Cemetery	2	26/06/2001	/	170/174	155/159	239/239	248/248	177/181
17	Nunhead Cemetery	2	26/06/2001	/	162/168	155/165	239/245	242/246	187/191
18	Nunhead Cemetery	2	26/06/2001	182/188	170/170	163/165	239/245	242/252	173/185
19	Nunhead Cemetery	2	26/06/2001	198/216	170/170	163/165	239/245	246/260	179/189
20	Nunhead Cemetery	2	26/06/2001	198/216	162/162	163/165	239/245	246/250	175/179
21	Nunhead Cemetery	3	26/06/2001	210/210	162/162	167/167	239/239	250/260	171/195
22	Nunhead Cemetery	3	26/06/2001	196/196	162/168	155/155	245/245	250/250	173/179
23	Nunhead Cemetery	3	26/06/2001	196/212	170/174	163/165	/	246/248	175/179
24	Nunhead Cemetery	3	26/06/2001	188/194	170/172	161/171	245/245	246/258	179/189
25	Nunhead Cemetery	3	26/06/2001	188/208	174/174	157/157	239/239	254/256	175/191
26	Nunhead Cemetery	3	26/06/2001	198/210	162/174	155/157	239/245	254/256	/
27	Nunhead Cemetery	3	26/06/2001	194/198	162/166	155/155	245/245	254/256	177/179
28	Nunhead Cemetery	3	26/06/2001	188/208	174/174	155/163	239/239	246/256	173/179
29	Nunhead Cemetery	3	26/06/2001	188/198	174/174	155/163	239/245	254/258	173/173
30	Nunhead Cemetery	3	26/06/2001	196/206	174/174	163/163	239/239	250/254	173/179
31	Nunhead Cemetery	4	26/06/2001	196/206	170/170	155/163	239/243	246/256	181/185
32	Nunhead Cemetery	4	26/06/2001	206/206	170/174	155/155	239/239	256/258	173/173
33	Nunhead Cemetery	4	26/06/2001	196/196	162/162	155/165	239/245	242/250	175/175
34	Nunhead Cemetery	4	26/06/2001	198/206	168/170	155/167	239/245	248/250	/
35	Nunhead Cemetery	4	26/06/2001	198/208	162/162	155/171	239/245	/	173/191
36	Nunhead Cemetery	4	26/06/2001	194/196	162/166	163/163	/	252/258	173/175
37	Nunhead Cemetery	4	26/06/2001	194/196	162/170	157/163	243/243	256/260	173/177
38	Nunhead Cemetery	4	26/06/2001	192/196	162/174	163/165	239/239	248/260	175/185
39	Nunhead Cemetery	4	26/06/2001	192/196	162/174	163/165	239/239	246/258	175/185
40	Nunhead Cemetery	4	26/06/2001	188/198	170/174	155/155	243/243	246/258	179/189
41	Nunhead Cemetery	5	26/06/2001	198/198	170/170	155/165	239/245	254/254	175/177
42	Nunhead Cemetery	5	26/06/2001	194/196	162/162	155/163	239/245	250/254	175/179
43	Nunhead Cemetery	5	26/06/2001	196/196	170/172	155/155	239/245	252/256	173/179
44	Nunhead Cemetery	5	26/06/2001	188/198	170/174	163/163	239/239	250/256	173/173
45	Nunhead Cemetery	5	26/06/2001	194/206	162/174	155/163	239/239	242/254	173/179
46	Nunhead Cemetery	5	26/06/2001	198/198	162/170	163/165	239/245	/	175/179

47	Nunhead Cemetery	5	26/06/2001	188/198	174/174	155/163	239/245	254/258	173/173
48	Nunhead Cemetery	5	26/06/2001	196/198	168/170	163/163	239/245	256/260	173/177
49	Nunhead Cemetery	5	26/06/2001	196/196	162/170	157/163	245/249	254/254	173/179
50	Nunhead Cemetery	5	12/07/2001	196/198	170/174	155/155	239/239	246/260	187/187
51	Nunhead Cemetery	6	12/07/2001	182/188	166/170	153/155	239/245	256/256	179/189
52	Nunhead Cemetery	6	12/07/2001	198/198	162/174	155/163	245/245	246/260	181/189
53	Nunhead Cemetery	6	12/07/2001	194/196	162/170	157/163	243/243	256/260	173/177
54	Nunhead Cemetery	6	12/07/2001	/	170/174	155/163	239/245	246/260	173/179
55	Nunhead Cemetery	6	12/07/2001	196/196	170/172	155/155	239/245	252/256	173/179
56	Nunhead Cemetery	6	12/07/2001	184/208	162/174	157/167	245/245	242/246	173/179
57	Nunhead Cemetery	6	12/07/2001	198/198	162/170	163/167	239/245	254/254	173/179
58	Nunhead Cemetery	6	12/07/2001	200/200	170/174	155/163	239/245	256/258	173/173
59	Nunhead Cemetery	6	12/07/2001	196/208	162/170	163/167	/	246/250	173/177
60	Nunhead Cemetery	6	12/07/2001	184/184	164/170	163/171	239/239	246/258	175/179
61	Nunhead Cemetery	7	12/07/2001	198/206	170/174	155/163	239/239	254/256	173/175
62	Nunhead Cemetery	7	12/07/2001	196/196	166/170	157/167	239/245	242/250	173/175
63	Nunhead Cemetery	7	12/07/2001	194/208	166/174	157/157	245/245	254/256	/
64	Nunhead Cemetery	7	12/07/2001	182/182	162/174	155/155	239/245	252/252	179/181
65	Nunhead Cemetery	7	12/07/2001	196/206	170/170	155/163	239/243	246/256	181/185
66	Nunhead Cemetery	7	12/07/2001	200/200	174/174	167/167	239/239	246/246	179/179
67	Nunhead Cemetery	7	12/07/2001	196/196	162/168	155/155	245/245	250/250	173/179
68	Nunhead Cemetery	7	12/07/2001	194/208	170/174	/	243/245	256/258	173/179
69	Nunhead Cemetery	7	12/07/2001	184/208	162/174	157/167	245/245	242/246	173/179
70	Nunhead Cemetery	7	12/07/2001	196/196	162/170	157/163	245/249	254/254	173/179
71	Nunhead Cemetery	8	12/07/2001	184/208	162/174	157/167	245/245	242/246	173/179
72	Nunhead Cemetery	8	12/07/2001	196/196	162/168	155/155	245/245	250/250	173/179
73	Nunhead Cemetery	8	12/07/2001	208/208	162/170	157/165	239/239	256/260	175/175
74	Nunhead Cemetery	8	12/07/2001	188/188	170/174	155/155	239/245	242/250	177/189
75	Nunhead Cemetery	8	12/07/2001	198/208	162/170	155/163	245/245	246/250	173/175
76	Nunhead Cemetery	8	12/07/2001	198/198	162/170	163/167	239/245	254/254	173/179
77	Nunhead Cemetery	8	12/07/2001	198/216	162/162	163/165	239/245	246/250	175/179
78	Nunhead Cemetery	8	12/07/2001	194/208	174/180	157/157	239/245	254/260	175/175
79	Nunhead Cemetery	8	12/07/2001	194/208	174/180	157/157	239/239	254/260	175/187
80	Nunhead Cemetery	8	12/07/2001	194/198	162/166	155/155	245/245	254/256	177/177
81	Nunhead Cemetery	9	12/07/2001	182/208	166/166	155/155	245/245	254/260	175/175
82	Nunhead Cemetery	9	12/07/2001	198/210	162/166	155/165	239/245	246/246	175/175
83	Nunhead Cemetery	9	12/07/2001	196/206	170/170	155/163	239/243	246/256	181/185
84	Nunhead Cemetery	9	12/07/2001	/	162/170	155/155	239/239	242/246	177/183
85	Nunhead Cemetery	9	12/07/2001	198/198	162/174	155/163	245/245	246/260	181/189
86	Nunhead Cemetery	9	12/07/2001	188/194	170/172	161/171	245/245	246/258	179/189
87	Nunhead Cemetery	9	12/07/2001	196/196	166/170	157/167	239/245	242/250	173/175
88	Nunhead Cemetery	9	12/07/2001	184/208	170/174	/	239/243	254/254	177/179
89	Nunhead Cemetery	9	12/07/2001	188/194	170/172	161/171	245/245	246/258	179/189
90	Nunhead Cemetery	9	12/07/2001	/	166/170	157/167	239/239	254/256	175/177
91	Nunhead Cemetery	10	12/07/2001	184/208	162/174	157/167	245/245	242/246	173/179
92	Nunhead Cemetery	10	12/07/2001	196/196	162/170	157/165	239/245	246/250	175/175
93	Nunhead Cemetery	10	12/07/2001	182/196	168/174	165/165	239/245	242/260	173/179
94	Nunhead Cemetery	10	12/07/2001	196/206	166/168	157/165	239/245	250/260	175/179
95	Nunhead Cemetery	10	12/07/2001	188/198	174/174	157/163	245/247	246/256	173/179
96	Nunhead Cemetery	10	12/07/2001	196/196	162/170	157/163	239/245	250/250	173/187
97	Nunhead Cemetery	10	12/07/2001	196/206	170/174	159/163	239/239	250/254	179/189
98	Nunhead Cemetery	10	12/07/2001	198/208	162/170	155/163	245/245	246/250	173/175

99	Nunhead Cemetery	10	12/07/2001	184/184	164/170	163/171	239/239	246/258	175/179
100	Nunhead Cemetery	10	12/07/2001	194/208	174/180	157/157	239/239	254/260	175/187
101	Barnes Common	11	03/07/2001	194/220	162/162	155/163	239/245	246/256	175/183
102	Barnes Common	11	03/07/2001	188/208	170/174	159/163	239/245	244/250	173/181
103	Barnes Common	11	03/07/2001	206/208	170/174	157/165	239/239	242/250	175/191
104	Barnes Common	11	03/07/2001	200/200	162/170	155/165	/	252/254	173/173
105	Barnes Common	11	03/07/2001	/	166/170	155/165	239/245	254/258	173/179
106	Barnes Common	11	03/07/2001	/	166/166	163/165	239/245	242/254	173/179
107	Barnes Common	11	03/07/2001	206/220	166/170	165/165	239/239	256/256	179/181
108	Barnes Common	11	03/07/2001	196/198	162/174	161/167	239/239	246/256	179/179
109	Barnes Common	11	03/07/2001	212/212	166/174	155/157	239/239	242/250	177/179
110	Barnes Common	11	03/07/2001	188/196	162/174	155/155	239/245	252/256	175/189
111	Barnes Common	12	03/07/2001	182/182	162/168	163/165	239/239	248/266	179/185
112	Barnes Common	12	03/07/2001	188/208	170/174	159/163	239/245	244/250	173/181
113	Barnes Common	12	03/07/2001	194/208	168/170	155/163	/	258/260	173/185
114	Barnes Common	12	03/07/2001	206/208	162/162	165/167	239/245	248/250	175/177
115	Barnes Common	12	03/07/2001	182/208	152/174	163/163	245/245	254/258	175/181
116	Barnes Common	12	03/07/2001	198/200	166/170	155/167	239/239	256/258	175/175
117	Barnes Common	12	03/07/2001	208/208	166/170	155/167	239/245	246/254	173/173
118	Barnes Common	12	03/07/2001	192/214	166/168	/	239/245	252/256	183/183
119	Barnes Common	12	03/07/2001	196/216	162/162	155/163	239/239	256/258	179/189
120	Barnes Common	12	03/07/2001	192/214	170/170	163/163	239/239	256/256	175/179
121	Barnes Common	13	03/07/2001	184/202	166/168	155/161	245/249	/	175/183
122	Barnes Common	13	03/07/2001	188/198	170/174	155/155	239/239	248/256	179/179
123	Barnes Common	13	03/07/2001	182/200	170/170	157/163	239/247	244/260	173/179
124	Barnes Common	13	03/07/2001	198/208	166/166	155/155	239/239	254/254	189/189
125	Barnes Common	13	03/07/2001	198/210	/	155/155	239/245	246/256	181/185
126	Barnes Common	13	03/07/2001	192/196	162/170	155/167	239/239	248/252	175/177
127	Barnes Common	13	03/07/2001	192/208	168/170	163/163	239/239	256/256	177/179
128	Barnes Common	13	03/07/2001	184/196	166/174	157/157	239/239	252/258	175/191
129	Barnes Common	13	03/07/2001	200/206	162/170	165/165	239/245	/	175/185
130	Barnes Common	13	03/07/2001	198/198	162/166	155/155	239/239	254/254	175/175
131	Barnes Common	14	03/07/2001	200/210	164/166	155/155	239/239	254/256	181/185
132	Barnes Common	14	03/07/2001	184/196	170/170	155/165	239/245	248/252	177/181
133	Barnes Common	14	03/07/2001	182/200	170/174	163/165	245/245	252/256	175/181
134	Barnes Common	14	03/07/2001	182/182	174/174	167/167	239/239	256/256	173/173
135	Barnes Common	14	03/07/2001	196/198	170/174	155/157	239/239	246/252	173/173
136	Barnes Common	14	03/07/2001	188/196	174/174	163/165	245/249	260/266	175/181
137	Barnes Common	14	03/07/2001	182/182	162/162	155/155	239/245	252/252	175/181
138	Barnes Common	14	03/07/2001	184/206	170/170	155/155	239/239	256/258	173/173
139	Barnes Common	14	03/07/2001	182/198	168/170	155/155	239/245	256/256	173/173
140	Barnes Common	14	03/07/2001	206/220	166/170	165/165	239/239	256/256	179/181
141	Barnes Common	15	03/07/2001	206/208	170/170	155/163	239/239	256/256	179/183
142	Barnes Common	15	03/07/2001	196/208	162/174	157/167	239/239	244/260	175/177
143	Barnes Common	15	03/07/2001	182/188	162/170	167/167	239/239	240/256	191/195
144	Barnes Common	15	03/07/2001	182/200	166/170	155/165	239/239	250/272	179/189
145	Barnes Common	15	03/07/2001	184/198	162/174	155/163	245/245	246/246	191/191
146	Barnes Common	15	03/07/2001	194/212	162/176	163/163	245/245	246/254	183/183
147	Barnes Common	15	03/07/2001	198/208	166/170	155/155	239/239	254/254	173/189
148	Barnes Common	15	03/07/2001	184/196	168/170	153/155	239/239	/	187/187
149	Barnes Common	15	03/07/2001	182/212	170/170	167/167	239/245	246/254	177/177
150	Barnes Common	15	03/07/2001	184/200	174/174	155/155	243/245	246/260	175/175

251	Barnes Common	16	27/06/2001	184/206	170/170	155/155	239/239	256/258	173/173
252	Barnes Common	16	27/06/2001	184/196	166/174	157/157	239/239	252/258	175/191
253	Barnes Common	16	27/06/2001	200/206	162/170	165/165	239/245	/	175/185
254	Barnes Common	16	27/06/2001	208/210	162/166	163/163	239/245	246/254	179/179
255	Barnes Common	16	27/06/2001	198/220	162/166	155/163	245/245	246/260	173/173
256	Barnes Common	16	27/06/2001	188/192	170/174	165/171	245/245	238/258	173/175
257	Barnes Common	16	27/06/2001	196/200	162/170	155/163	/	252/254	173/175
258	Barnes Common	16	27/06/2001	188/188	174/180	157/163	239/245	248/252	173/175
259	Barnes Common	16	27/06/2001	194/196	162/174	163/167	239/245	252/258	183/201
260	Barnes Common	16	27/06/2001	200/210	162/174	163/163	239/239	248/258	173/173
261	Barnes Common	17	27/06/2001	198/200	162/162	163/163	245/245	256/256	179/179
262	Barnes Common	17	27/06/2001	194/208	162/168	155/161	239/243	256/256	177/179
263	Barnes Common	17	27/06/2001	188/198	/	155/163	243/245	252/252	173/175
264	Barnes Common	17	27/06/2001	182/194	170/174	155/163	/	250/250	179/179
265	Barnes Common	17	27/06/2001	188/192	170/170	163/163	239/239	252/252	177/177
266	Barnes Common	17	27/06/2001	196/198	162/170	155/155	245/245	242/248	187/191
267	Barnes Common	17	27/06/2001	182/182	162/174	155/163	245/245	248/260	171/195
268	Barnes Common	17	27/06/2001	182/182	162/162	155/155	239/245	252/252	175/181
269	Barnes Common	17	27/06/2001	196/198	174/174	159/163	239/247	246/256	175/191
270	Barnes Common	17	27/06/2001	198/220	162/166	155/163	245/245	246/260	173/173
271	Barnes Common	18	27/06/2001	182/212	162/174	163/167	245/245	248/260	171/173
272	Barnes Common	18	27/06/2001	194/196	170/172	155/161	/	258/258	177/179
273	Barnes Common	18	27/06/2001	188/194	162/170	155/155	239/245	250/256	173/179
274	Barnes Common	18	27/06/2001	188/198	170/174	155/155	239/239	248/256	179/179
275	Barnes Common	18	27/06/2001	188/208	170/174	159/163	239/245	244/250	173/181
276	Barnes Common	18	27/06/2001	192/208	168/170	163/163	239/239	256/256	177/179
277	Barnes Common	18	27/06/2001	182/208	162/174	163/165	239/245	254/258	179/179
278	Barnes Common	18	27/06/2001	188/198	170/174	155/155	239/239	248/256	179/179
279	Barnes Common	18	27/06/2001	196/216	162/162	155/163	239/239	256/258	179/189
280	Barnes Common	18	27/06/2001	192/196	162/170	155/167	239/239	248/252	175/177
281	Barnes Common	19	27/06/2001	192/206	162/174	157/165	239/245	250/260	173/173
282	Barnes Common	19	27/06/2001	194/208	162/168	155/161	239/243	256/256	177/179
283	Barnes Common	19	27/06/2001	188/198	170/174	155/155	239/239	248/256	179/179
284	Barnes Common	19	27/06/2001	194/220	162/162	155/163	239/245	246/256	175/183
285	Barnes Common	19	27/06/2001	198/198	168/170	157/165	239/247	252/256	175/189
286	Barnes Common	19	27/06/2001	204/226	162/174	157/163	239/245	256/256	173/179
287	Barnes Common	19	27/06/2001	182/206	170/174	155/165	239/245	246/268	175/189
288	Barnes Common	19	27/06/2001	182/182	162/162	155/155	239/245	252/252	175/181
289	Barnes Common	19	27/06/2001	184/208	170/174	155/167	245/245	238/256	173/173
290	Barnes Common	19	27/06/2001	188/194	162/170	163/165	245/245	250/256	175/175
291	Barnes Common	20	27/06/2001	194/194	170/174	155/155	239/245	256/256	183/191
292	Barnes Common	20	27/06/2001	194/220	162/162	155/163	239/245	246/256	175/183
293	Barnes Common	20	27/06/2001	188/208	170/174	159/163	239/245	244/250	173/181
294	Barnes Common	20	27/06/2001	/	/	/	/	/	/
295	Barnes Common	20	27/06/2001	196/216	162/162	155/163	239/239	256/258	179/189
296	Barnes Common	20	27/06/2001	196/208	162/174	155/163	239/247	/	175/185
297	Barnes Common	20	27/06/2001	194/206	162/170	157/165	239/245	252/262	173/175
298	Barnes Common	20	27/06/2001	198/198	162/162	155/155	239/245	258/260	175/177
299	Barnes Common	20	27/06/2001	184/196	166/174	157/157	239/239	252/258	175/191
300	Barnes Common	20	27/06/2001	196/216	166/174	157/167	245/249	252/254	173/179
501	Regent's Park	21	20/07/2001	182/190	172/174	159/165	239/245	252/254	173/179
502	Regent's Park	21	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181

503	Regent's Park	21	20/07/2001	188/188	170/170	155/155	245/245	256/258	179/179
504	Regent's Park	21	20/07/2001	/	/	/	/	/	/
505	Regent's Park	21	20/07/2001	194/212	170/170	163/167	245/245	252/254	173/173
506	Regent's Park	21	20/07/2001	188/188	170/170	155/155	245/245	256/258	179/179
507	Regent's Park	21	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
508	Regent's Park	21	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
509	Regent's Park	21	20/07/2001	/	/	/	/	/	/
510	Regent's Park	21	20/07/2001	/	/	/	/	/	/
511	Regent's Park	22	20/07/2001	196/212	162/174	163/165	239/245	256/258	173/191
512	Regent's Park	22	20/07/2001	198/210	162/174	157/157	239/239	250/250	173/191
513	Regent's Park	22	20/07/2001	182/190	172/174	159/165	239/245	252/254	173/179
514	Regent's Park	22	20/07/2001	/	/	/	/	/	/
515	Regent's Park	22	20/07/2001	/	/	/	/	/	/
516	Regent's Park	22	20/07/2001	182/190	172/174	159/165	239/245	252/254	173/179
517	Regent's Park	22	20/07/2001	194/200	170/170	155/163	239/245	242/250	179/185
518	Regent's Park	22	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
519	Regent's Park	22	20/07/2001	194/200	170/170	155/163	239/245	242/250	179/185
520	Regent's Park	22	20/07/2001	196/208	168/174	157/163	243/245	250/260	179/195
521	Regent's Park	23	20/07/2001	196/210	170/174	155/165	239/239	246/256	189/191
522	Regent's Park	23	20/07/2001	/	/	/	/	/	/
523	Regent's Park	23	20/07/2001	/	/	/	/	/	/
524	Regent's Park	23	20/07/2001	196/200	172/174	155/167	245/245	254/254	183/189
525	Regent's Park	23	20/07/2001	194/212	170/170	163/167	245/245	252/254	173/173
526	Regent's Park	23	20/07/2001	196/198	162/166	163/167	245/249	258/258	173/179
527	Regent's Park	23	20/07/2001	198/216	170/176	155/165	239/245	/	173/173
528	Regent's Park	23	20/07/2001	182/198	170/176	155/155	239/239	250/250	173/179
529	Regent's Park	23	20/07/2001	/	/	/	/	/	/
530	Regent's Park	23	20/07/2001	/	/	/	/	/	/
531	Regent's Park	24	20/07/2001	188/188	170/170	155/155	245/245	256/258	179/179
532	Regent's Park	24	20/07/2001	/	/	/	/	/	/
533	Regent's Park	24	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
534	Regent's Park	24	20/07/2001	194/200	170/170	155/163	239/245	242/250	179/185
535	Regent's Park	24	20/07/2001	/	/	/	/	/	/
536	Regent's Park	24	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
537	Regent's Park	24	20/07/2001	182/198	170/170	155/155	239/239	250/250	173/179
538	Regent's Park	24	20/07/2001	198/200	162/162	157/163	239/247	258/260	175/187
539	Regent's Park	24	20/07/2001	196/208	168/174	157/163	243/245	250/260	179/195
540	Regent's Park	24	20/07/2001	196/210	170/174	155/165	239/239	246/256	189/191
541	Regent's Park	25	20/07/2001	194/212	170/170	163/167	245/245	252/254	173/173
542	Regent's Park	25	20/07/2001	184/202	160/160	155/167	239/245	250/254	173/179
543	Regent's Park	25	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
544	Regent's Park	25	20/07/2001	194/212	170/170	163/167	245/245	252/254	173/173
545	Regent's Park	25	20/07/2001	/	/	/	/	/	/
546	Regent's Park	25	20/07/2001	/	/	/	/	/	/
547	Regent's Park	25	20/07/2001	198/210	162/174	157/157	239/239	250/250	173/191
548	Regent's Park	25	20/07/2001	194/198	162/170	155/167	239/245	252/258	175/175
549	Regent's Park	25	20/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
550	Regent's Park	25	20/07/2001	196/210	164/166	155/163	239/245	250/256	173/181
551	Regent's Park	26	21/07/2001	/	/	/	/	/	/
552	Regent's Park	26	21/07/2001	196/212	162/170	155/165	239/245	238/258	179/181

553	Regent's Park	26	21/07/2001	182/190	172/174	159/165	239/245	252/254	173/179
554	Regent's Park	26	21/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
555	Regent's Park	26	21/07/2001	196/208	168/174	157/163	243/245	250/260	179/195
556	Regent's Park	26	21/07/2001	/	/	/	/	/	/
557	Regent's Park	26	21/07/2001	/	/	/	/	/	/
558	Regent's Park	26	21/07/2001	182/198	170/170	155/155	239/239	250/250	173/179
559	Regent's Park	26	21/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
560	Regent's Park	26	21/07/2001	196/208	168/174	157/163	243/245	250/260	179/195
561	Regent's Park	27	21/07/2001	196/210	170/174	155/165	239/239	246/256	189/191
562	Regent's Park	27	21/07/2001	188/188	170/170	155/155	245/245	256/258	179/179
563	Regent's Park	27	21/07/2001	/	/	/	/	/	/
564	Regent's Park	27	21/07/2001	/	/	/	/	/	/
565	Regent's Park	27	21/07/2001	/	/	/	/	/	/
566	Regent's Park	27	21/07/2001	196/208	168/174	157/163	243/245	250/260	179/195
567	Regent's Park	27	21/07/2001	/	/	/	/	/	/
568	Regent's Park	27	21/07/2001	/	/	/	/	/	/
569	Regent's Park	27	21/07/2001	/	/	/	/	/	/
570	Regent's Park	27	21/07/2001	196/208	168/174	157/163	243/245	250/260	179/195
571	Regent's Park	28	21/07/2001	206/212	162/162	155/165	239/245	246/250	/
572	Regent's Park	28	21/07/2001	200/212	166/170	155/163	239/245	/	173/179
573	Regent's Park	28	21/07/2001	196/210	170/174	155/165	239/239	246/256	189/191
574	Regent's Park	28	21/07/2001	182/206	166/174	163/165	239/245	/	173/185
575	Regent's Park	28	21/07/2001	/	/	/	/	/	/
576	Regent's Park	28	21/07/2001	/	/	/	/	/	/
577	Regent's Park	28	21/07/2001	/	/	/	/	/	/
578	Regent's Park	28	21/07/2001	/	/	/	/	/	/
579	Regent's Park	28	21/07/2001	196/212	162/170	155/165	239/245	238/258	179/181
580	Regent's Park	28	21/07/2001	206/212	162/162	155/165	239/245	246/250	/
581	Regent's Park	29	21/07/2001	182/200	170/174	157/163	239/239	246/246	179/189
582	Regent's Park	29	21/07/2001	182/200	170/174	155/155	245/245	/	173/173
583	Regent's Park	29	21/07/2001	188/188	170/170	157/163	239/239	/	173/177
584	Regent's Park	29	21/07/2001	182/196	170/170	157/163	239/239	258/258	173/183
585	Regent's Park	29	21/07/2001	194/208	166/170	155/163	239/245	250/256	175/185
586	Regent's Park	29	21/07/2001	182/196	162/174	163/163	239/245	246/256	173/177
587	Regent's Park	29	21/07/2001	188/210	170/174	157/167	245/245	246/256	173/189
588	Regent's Park	29	21/07/2001	184/202	162/170	155/163	239/245	/	173/187
589	Regent's Park	29	21/07/2001	196/208	162/168	157/163	239/239	/	173/179
590	Regent's Park	29	21/07/2001	188/198	168/170	155/165	245/245	/	173/179
591	Regent's Park	30	21/07/2001	194/198	170/174	155/163	239/245	246/256	173/173
592	Regent's Park	30	21/07/2001	196/210	164/166	155/163	239/245	250/256	173/181
593	Regent's Park	30	21/07/2001	196/208	168/174	157/163	243/245	250/260	179/195
594	Regent's Park	30	21/07/2001	196/198	162/166	163/167	245/249	258/258	173/179
595	Regent's Park	30	21/07/2001	182/198	174/176	157/163	239/239	/	183/185
596	Regent's Park	30	21/07/2001	182/198	174/176	157/163	239/239	254/254	183/183
597	Regent's Park	30	21/07/2001	206/208	174/174	155/165	239/245	246/256	175/179
598	Regent's Park	30	21/07/2001	194/196	168/170	157/163	239/245	256/258	173/183
599	Regent's Park	30	21/07/2001	196/210	170/174	155/165	239/239	246/256	189/191
600	Regent's Park	30	21/07/2001	196/208	170/170	155/163	239/245	250/256	179/185
701	Millenium Village	31	27/07/2001	194/194	162/166	155/163	245/245	254/254	181/189
702	Millenium Village	31	27/07/2001	182/212	170/174	155/157	239/245	256/258	173/183

703	Millenium Village	31	27/07/2001	206/206	162/186	163/165	239/245	246/250	173/173
704	Millenium Village	31	27/07/2001	194/198	166/170	159/163	239/239	252/256	173/181
705	Millenium Village	31	27/07/2001	182/184	162/166	155/155	239/245	256/256	173/175
706	Millenium Village	31	27/07/2001	196/210	170/174	157/163	245/245	246/254	179/179
707	Millenium Village	31	27/07/2001	196/210	162/162	155/165	239/245	254/254	173/179
708	Millenium Village	31	27/07/2001	182/182	170/170	159/165	239/239	246/256	175/175
709	Millenium Village	31	27/07/2001	182/196	162/162	155/163	239/245	246/256	173/185
710	Millenium Village	31	27/07/2001	182/182	164/170	155/165	239/243	/	177/189
711	Millenium Village	32	27/07/2001	188/194	170/170	155/163	239/245	258/258	173/173
712	Millenium Village	32	27/07/2001	182/198	174/174	157/171	239/245	246/246	191/191
713	Millenium Village	32	27/07/2001	200/206	162/170	165/171	239/245	/	175/179
714	Millenium Village	32	27/07/2001	196/198	162/162	155/167	239/247	256/256	173/173
715	Millenium Village	32	27/07/2001	200/208	166/168	155/163	/	242/252	173/173
716	Millenium Village	32	27/07/2001	196/200	170/174	163/163	239/245	246/266	179/185
717	Millenium Village	32	27/07/2001	200/212	174/174	155/163	245/245	/	179/189
718	Millenium Village	32	27/07/2001	194/198	168/174	163/171	239/245	254/256	173/173
719	Millenium Village	32	27/07/2001	184/196	174/174	165/171	245/245	242/246	189/189
720	Millenium Village	32	27/07/2001	202/202	160/166	163/167	237/243	252/252	175/175
721	Millenium Village	33	27/07/2001	198/216	162/170	155/167	239/239	/	201/201
722	Millenium Village	33	27/07/2001	182/196	/	155/155	239/245	254/256	173/191
723	Millenium Village	33	27/07/2001	194/198	170/174	157/157	239/247	248/248	175/179
724	Millenium Village	33	27/07/2001	200/216	162/174	155/163	245/245	250/260	179/179
725	Millenium Village	33	27/07/2001	198/216	170/170	163/163	239/239	248/256	173/187
726	Millenium Village	33	27/07/2001	/	168/168	163/165	239/245	254/260	173/179
727	Millenium Village	33	27/07/2001	188/202	162/174	155/163	245/245	258/260	173/187
728	Millenium Village	33	27/07/2001	198/208	170/174	157/163	239/239	/	179/179
729	Millenium Village	33	27/07/2001	194/198	166/170	155/163	239/239	252/256	173/179
730	Millenium Village	33	27/07/2001	208/216	160/168	155/165	/	242/254	173/185
731	Millenium Village	34	27/07/2001	184/192	162/168	155/155	239/239	254/260	179/179
732	Millenium Village	34	27/07/2001	194/210	162/162	149/167	245/245	258/260	179/179
733	Millenium Village	34	27/07/2001	182/184	162/166	155/155	239/239	254/260	175/175
734	Millenium Village	34	27/07/2001	182/194	162/170	159/163	239/247	252/260	175/175
735	Millenium Village	34	27/07/2001	202/202	160/166	165/167	233/235	238/242	/
736	Millenium Village	34	27/07/2001	208/208	170/170	155/155	243/245	250/260	175/177
737	Millenium Village	34	27/07/2001	208/212	170/170	155/155	239/245	/	175/179
738	Millenium Village	34	27/07/2001	194/198	170/174	165/165	239/239	242/254	/
739	Millenium Village	34	27/07/2001	188/198	168/170	155/155	/	254/256	175/175
740	Millenium Village	34	27/07/2001	182/196	162/174	163/171	239/239	246/256	173/185
741	Millenium Village	35	27/07/2001	196/202	166/166	163/167	237/243	252/252	175/175
742	Millenium Village	35	27/07/2001	196/208	170/174	157/171	239/245	246/256	179/179
743	Millenium Village	35	27/07/2001	198/198	168/168	163/163	239/247	256/256	173/189
744	Millenium Village	35	27/07/2001	198/216	170/170	163/163	239/239	248/256	173/187
745	Millenium Village	35	27/07/2001	184/200	166/170	155/165	245/245	242/252	175/175
746	Millenium Village	35	27/07/2001	182/194	170/170	155/163	239/239	/	177/177
747	Millenium Village	35	27/07/2001	206/206	162/162	155/163	239/245	246/250	173/173
748	Millenium Village	35	27/07/2001	194/208	166/170	155/163	239/245	252/260	175/185
749	Millenium Village	35	27/07/2001	182/208	162/162	155/155	239/239	246/252	173/173
750	Millenium Village	35	27/07/2001	182/194	170/174	155/155	239/245	238/246	179/179
751	Millenium Village	36	30/07/2001	208/208	162/174	155/167	239/239	252/252	189/195
752	Millenium Village	36	30/07/2001	182/196	162/174	155/163	239/239	246/256	173/185

753	Millenium Village	36	30/07/2001	200/206	160/172	157/161	239/245	256/256	183/185
754	Millenium Village	36	30/07/2001	194/198	170/174	155/157	239/239	/	175/177
755	Millenium Village	36	30/07/2001	208/208	162/162	155/163	239/239	242/252	173/173
756	Millenium Village	36	30/07/2001	182/194	166/170	155/163	/	250/254	175/179
757	Millenium Village	36	30/07/2001	184/208	162/166	155/163	245/245	242/252	177/189
758	Millenium Village	36	30/07/2001	208/212	170/170	155/163	243/245	250/260	175/177
759	Millenium Village	36	30/07/2001	188/198	162/168	163/163	239/245	246/258	/
760	Millenium Village	36	30/07/2001	194/198	166/170	155/163	239/239	252/256	173/179
761	Millenium Village	37	30/07/2001	198/200	162/162	163/165	239/245	246/250	173/177
762	Millenium Village	37	30/07/2001	184/208	162/166	155/163	245/245	242/252	177/177
763	Millenium Village	37	30/07/2001	188/218	162/168	165/171	/	248/252	191/201
764	Millenium Village	37	30/07/2001	184/208	162/166	163/163	245/245	242/252	173/173
765	Millenium Village	37	30/07/2001	194/208	166/170	155/163	239/245	252/260	175/185
766	Millenium Village	37	30/07/2001	208/212	170/170	155/155	239/245	242/258	175/179
767	Millenium Village	37	30/07/2001	192/198	162/168	155/157	/	254/256	173/179
768	Millenium Village	37	30/07/2001	200/200	160/172	157/161	239/245	256/258	183/185
769	Millenium Village	37	30/07/2001	194/198	168/174	163/171	239/245	254/256	173/173
770	Millenium Village	37	30/07/2001	184/196	166/170	155/155	245/245	/	173/175
771	Millenium Village	38	30/07/2001	206/206	164/166	163/163	239/245	/	175/177
772	Millenium Village	38	30/07/2001	206/206	170/174	155/155	239/245	246/256	181/181
773	Millenium Village	38	30/07/2001	188/206	162/162	165/165	239/245	250/254	/
774	Millenium Village	38	30/07/2001	188/194	162/170	155/165	239/245	258/258	173/179
775	Millenium Village	38	30/07/2001	198/200	162/168	157/167	239/247	256/256	173/173
776	Millenium Village	38	30/07/2001	196/200	170/174	163/163	239/245	246/266	179/185
777	Millenium Village	38	30/07/2001	190/206	164/166	155/163	239/245	/	173/175
778	Millenium Village	38	30/07/2001	194/198	170/174	157/157	239/247	248/248	175/179
779	Millenium Village	38	30/07/2001	198/200	162/162	163/165	239/245	246/250	173/173
780	Millenium Village	38	30/07/2001	/	160/168	155/167	239/245	/	171/173
781	Millenium Village	39	30/07/2001	182/210	160/166	159/165	239/245	/	173/183
782	Millenium Village	39	30/07/2001	182/188	162/174	155/155	245/245	/	173/175
783	Millenium Village	39	30/07/2001	208/208	162/168	157/167	239/239	246/252	173/173
784	Millenium Village	39	30/07/2001	194/208	166/170	155/163	239/245	252/260	175/185
785	Millenium Village	39	30/07/2001	208/212	170/170	155/155	239/245	242/258	175/179
786	Millenium Village	39	30/07/2001	182/196	162/162	163/167	239/245	252/258	173/179
787	Millenium Village	39	30/07/2001	196/208	166/174	157/167	239/245	256/260	191/195
788	Millenium Village	39	30/07/2001	188/196	162/174	155/163	239/243	254/260	173/173
789	Millenium Village	39	30/07/2001	192/196	164/166	155/165	239/239	/	177/185
790	Millenium Village	39	30/07/2001	194/198	160/170	163/163	245/245	246/254	173/175
791	Millenium Village	40	30/07/2001	206/206	170/174	155/155	239/245	246/256	181/181
792	Millenium Village	40	30/07/2001	194/196	166/170	157/163	/	246/250	173/173
793	Millenium Village	40	30/07/2001	194/196	166/170	163/163	239/245	246/254	173/173
794	Millenium Village	40	30/07/2001	194/198	170/174	165/165	239/239	242/254	/
795	Millenium Village	40	30/07/2001	182/208	162/170	163/163	239/239	256/260	179/191
796	Millenium Village	40	30/07/2001	198/198	162/162	155/165	239/245	250/256	173/181
797	Millenium Village	40	30/07/2001	188/198	170/174	155/163	239/239	246/256	179/191
798	Millenium Village	40	30/07/2001	196/208	162/168	163/165	239/245	250/250	173/189
799	Millenium Village	40	30/07/2001	188/194	166/170	157/163	239/245	246/254	173/173
800	Millenium Village	40	30/07/2001	196/208	166/174	157/167	/	252/258	191/195
921	Thames Barrier	41	05/07/2001	210/212	170/174	155/157	239/245	246/254	175/177

922	Thames Barrier	41	05/07/2001	206/206	170/170	167/167	239/245	254/256	175/177
923	Thames Barrier	41	05/07/2001	184/206	162/166	155/167	239/245	252/256	175/175
924	Thames Barrier	41	05/07/2001	198/200	166/174	155/165	239/239	246/256	179/181
925	Thames Barrier	41	05/07/2001	194/196	162/170	155/155	239/239	246/256	191/191
926	Thames Barrier	41	05/07/2001	196/196	162/172	155/163	245/245	246/254	177/177
927	Thames Barrier	41	05/07/2001	194/198	162/170	157/163	245/245	254/260	175/175
928	Thames Barrier	41	05/07/2001	182/212	170/174	155/163	239/245	254/258	175/177
929	Thames Barrier	41	05/07/2001	182/188	162/174	171/171	239/245	256/256	175/181
930	Thames Barrier	41	05/07/2001	182/200	162/170	155/155	245/245	250/254	175/175
931	Thames Barrier	42	05/07/2001	196/200	162/174	155/163	239/245	246/246	175/179
932	Thames Barrier	42	05/07/2001	196/206	170/174	155/163	245/245	246/256	177/185
933	Thames Barrier	42	05/07/2001	206/208	162/170	155/167	239/239	246/246	175/181
934	Thames Barrier	42	05/07/2001	198/198	162/162	155/163	239/245	246/260	177/181
935	Thames Barrier	42	05/07/2001	198/200	162/170	155/163	239/245	256/260	181/189
936	Thames Barrier	42	05/07/2001	198/208	162/166	163/171	239/245	256/256	175/175
937	Thames Barrier	42	05/07/2001	182/188	162/170	157/165	243/245	246/246	175/181
938	Thames Barrier	42	05/07/2001	196/196	170/174	157/165	239/239	254/256	175/181
939	Thames Barrier	42	05/07/2001	198/198	166/170	155/163	243/245	256/256	175/177
940	Thames Barrier	42	05/07/2001	182/212	170/170	157/165	239/245	254/256	175/175
941	Thames Barrier	43	05/07/2001	196/208	170/174	155/165	239/245	254/256	177/181
942	Thames Barrier	43	05/07/2001	188/206	168/174	155/155	239/239	256/256	175/175
943	Thames Barrier	43	05/07/2001	194/196	170/174	165/167	239/245	246/256	177/181
944	Thames Barrier	43	05/07/2001	182/184	170/170	155/165	245/245	246/248	179/179
945	Thames Barrier	43	05/07/2001	194/194	170/170	155/157	239/245	242/260	175/183
946	Thames Barrier	43	05/07/2001	188/206	162/170	155/155	245/247	242/256	175/181
947	Thames Barrier	43	05/07/2001	198/198	162/166	155/163	243/245	246/250	175/177
948	Thames Barrier	43	05/07/2001	196/196	162/170	155/165	239/245	254/256	175/175
949	Thames Barrier	43	05/07/2001	196/208	174/176	155/167	239/239	242/256	177/181
950	Thames Barrier	43	05/07/2001	196/210	166/174	155/155	239/239	254/256	175/175
951	Thames Barrier	44	05/07/2001	208/208	166/168	163/171	239/245	250/256	175/175
952	Thames Barrier	44	05/07/2001	182/208	162/170	155/165	239/239	242/250	179/179
953	Thames Barrier	44	05/07/2001	192/208	162/168	155/165	239/245	246/250	181/183
954	Thames Barrier	44	05/07/2001	194/208	168/168	163/165	239/245	250/254	181/189
955	Thames Barrier	44	05/07/2001	196/198	162/162	155/163	251/251	254/254	177/183
956	Thames Barrier	44	05/07/2001	196/196	162/170	163/167	239/245	246/256	181/189
957	Thames Barrier	44	05/07/2001	188/196	170/170	155/167	/	250/256	175/175
958	Thames Barrier	44	05/07/2001	206/208	162/162	167/167	/	254/262	175/175
959	Thames Barrier	44	05/07/2001	182/182	162/170	155/167	239/245	260/260	175/175
960	Thames Barrier	44	05/07/2001	196/196	170/174	155/165	245/245	250/256	189/191
961	Thames Barrier	45	05/07/2001	196/208	170/170	155/155	239/245	248/264	175/177
962	Thames Barrier	45	05/07/2001	182/198	162/174	155/163	/	246/250	175/175
963	Thames Barrier	45	05/07/2001	196/196	170/170	155/167	239/245	242/254	175/181
964	Thames Barrier	45	05/07/2001	196/198	170/170	155/167	245/245	256/258	175/181
965	Thames Barrier	45	05/07/2001	182/198	168/170	149/155	251/251	250/250	177/177
966	Thames Barrier	45	05/07/2001	196/206	/	155/165	245/245	246/252	175/175
967	Thames Barrier	45	05/07/2001	182/198	162/174	157/163	239/245	246/256	175/175
968	Thames Barrier	45	05/07/2001	196/206	170/174	155/163	239/245	252/252	175/175
969	Thames Barrier	45	05/07/2001	194/196	162/170	155/155	245/245	246/258	175/181
970	Thames Barrier	45	05/07/2001	196/212	162/170	/	255/255	246/260	175/181
971	Thames Barrier	46	05/07/2001	198/198	162/170	155/155	245/245	250/254	/
972	Thames Barrier	46	05/07/2001	192/216	162/162	163/171	255/255	256/256	177/179
973	Thames Barrier	46	05/07/2001	198/216	162/162	155/163	/	256/260	183/183

974	Thames Barrier	46	05/07/2001	208/216	166/170	/	239/239	246/254	177/177
975	Thames Barrier	46	05/07/2001	200/212	162/170	155/163	239/245	246/254	181/195
976	Thames Barrier	46	05/07/2001	182/210	170/174	155/165	239/239	250/260	191/191
977	Thames Barrier	46	05/07/2001	182/184	168/174	161/161	239/245	260/260	181/181
978	Thames Barrier	46	05/07/2001	194/208	162/166	155/167	239/251	254/256	179/179
979	Thames Barrier	46	05/07/2001	184/188	162/170	155/167	239/239	246/260	175/181
980	Thames Barrier	46	05/07/2001	/	/	/	/	/	/
981	Thames Barrier	47	05/07/2001	188/210	162/174	155/163	239/245	254/256	175/177
982	Thames Barrier	47	05/07/2001	188/196	170/170	157/163	239/239	254/260	175/185
983	Thames Barrier	47	05/07/2001	206/212	170/174	167/167	239/239	254/254	175/177
984	Thames Barrier	47	05/07/2001	194/198	172/174	155/167	239/245	254/256	175/177
985	Thames Barrier	47	05/07/2001	198/208	170/174	157/157	251/257	246/252	181/181
986	Thames Barrier	47	05/07/2001	196/198	162/170	155/167	239/245	246/260	181/183
987	Thames Barrier	47	05/07/2001	198/200	162/174	/	251/257	258/258	187/191
988	Thames Barrier	47	05/07/2001	/	168/174	163/165	245/245	256/256	177/191
989	Thames Barrier	47	05/07/2001	196/206	170/174	163/165	245/245	256/256	175/181
990	Thames Barrier	47	05/07/2001	200/200	162/170	155/163	245/245	256/256	181/191
991	Thames Barrier	48	05/07/2001	188/208	170/170	155/155	239/245	234/256	181/187
992	Thames Barrier	48	05/07/2001	198/206	162/166	163/163	239/239	246/256	177/183
993	Thames Barrier	48	05/07/2001	196/206	162/174	161/165	239/245	238/238	181/189
994	Thames Barrier	48	05/07/2001	182/194	170/174	155/161	239/245	246/250	175/175
995	Thames Barrier	48	05/07/2001	196/208	162/170	155/163	239/245	250/256	175/175
996	Thames Barrier	48	05/07/2001	182/212	170/170	163/167	239/239	256/256	175/187
997	Thames Barrier	48	05/07/2001	/	/	/	/	/	/
998	Thames Barrier	48	05/07/2001	194/198	162/168	155/157	239/245	246/256	175/181
999	Thames Barrier	48	05/07/2001	206/212	/	165/167	245/247	256/260	175/175
1000	Thames Barrier	48	05/07/2001	196/198	162/170	155/165	239/245	238/256	177/179
1001	Thames Barrier	49	05/07/2001	194/206	162/174	149/163	245/245	256/260	179/183
1002	Thames Barrier	49	05/07/2001	196/216	162/174	155/163	245/245	246/256	175/179
1003	Thames Barrier	49	05/07/2001	196/216	162/170	155/155	245/245	246/254	175/177
1004	Thames Barrier	49	05/07/2001	198/198	162/174	155/165	239/245	246/256	175/181
1005	Thames Barrier	49	05/07/2001	212/212	170/174	155/163	239/239	242/256	175/175
1006	Thames Barrier	49	05/07/2001	182/182	162/162	163/163	245/247	246/260	175/181
1007	Thames Barrier	49	05/07/2001	194/194	168/170	/	245/245	246/256	175/177
1008	Thames Barrier	49	05/07/2001	190/200	160/168	155/163	245/245	256/260	187/197
1009	Thames Barrier	49	05/07/2001	194/208	162/168	165/167	239/245	256/260	175/179
1010	Thames Barrier	49	05/07/2001	182/198	162/162	155/163	239/239	250/256	177/181
1011	Thames Barrier	50	05/07/2001	196/196	162/174	157/157	239/239	246/256	175/175
1012	Thames Barrier	50	05/07/2001	188/200	170/170	155/155	239/245	246/250	175/175
1013	Thames Barrier	50	05/07/2001	196/198	170/174	155/155	239/239	254/264	175/177
1014	Thames Barrier	50	05/07/2001	196/196	170/174	163/163	245/245	256/260	175/177
1015	Thames Barrier	50	05/07/2001	196/196	168/178	155/155	239/245	250/256	175/175
1016	Thames Barrier	50	05/07/2001	196/196	168/178	155/155	239/245	250/256	175/175
1017	Thames Barrier	50	05/07/2001	192/200	170/170	155/163	239/239	242/246	179/179
1018	Thames Barrier	50	05/07/2001	182/182	162/172	155/165	245/245	246/256	179/179
1019	Thames Barrier	50	05/07/2001	198/208	170/174	165/165	245/245	250/256	175/175
1020	Thames Barrier	50	05/07/2001	200/208	162/170	155/163	/	254/260	179/191
801	Tolworth	51	21/06/2001	198/206	162/174	/	245/245	248/252	179/191
802	Tolworth	51	21/06/2001	/	162/174	155/155	239/245	252/256	173/181
803	Tolworth	51	21/06/2001	196/198	170/174	155/171	239/239	246/254	173/179
804	Tolworth	51	21/06/2001	/	160/160	163/165	/	246/254	/
805	Tolworth	51	21/06/2001	182/188	162/170	155/155	239/247	242/252	173/175

806	Tolworth	51	21/06/2001	/	162/166	155/163	245/245	254/256	179/179
807	Tolworth	51	21/06/2001	196/198	170/174	155/171	239/239	254/254	173/179
808	Tolworth	51	21/06/2001	182/196	174/174	155/163	239/247	246/260	/
809	Tolworth	51	21/06/2001	182/182	166/170	155/163	245/245	248/252	173/173
810	Tolworth	51	21/06/2001	182/196	174/174	155/163	239/247	246/258	/
811	Hanwell	52	21/06/2001	182/182	162/170	/	239/239	/	179/189
812	Hanwell	52	21/06/2001	196/196	170/170	155/163	239/239	246/256	175/189
813	Hanwell	52	21/06/2001	196/198	162/166	155/167	239/239	246/256	173/185
814	Hanwell	52	21/06/2001	198/200	174/174	161/161	243/243	258/258	191/191
815	Hanwell	52	21/06/2001	208/208	162/174	163/163	239/245	254/264	179/179
816	Hanwell	52	21/06/2001	/	170/170	165/165	245/245	260/260	179/179
817	Hanwell	52	21/06/2001	188/206	162/170	149/155	245/245	254/256	173/175
818	Hanwell	52	21/06/2001	188/206	162/170	149/155	245/245	250/254	/
819	Hanwell	52	21/06/2001	206/208	170/174	155/155	239/245	254/254	179/183
820	Hanwell	52	21/06/2001	208/208	162/162	163/163	245/245	254/264	177/177
831	Woodgrange Park	53	22/06/2001	192/194	166/170	155/163	239/245	256/256	175/175
832	Woodgrange Park	53	22/06/2001	184/198	174/174	155/155	243/245	/	179/179
833	Woodgrange Park	53	22/06/2001	182/208	170/174	163/163	245/245	254/268	/
834	Woodgrange Park	53	22/06/2001	182/196	170/174	155/159	239/245	242/252	173/191
835	Woodgrange Park	53	22/06/2001	182/206	170/170	157/163	245/245	246/254	185/187
836	Woodgrange Park	53	22/06/2001	188/194	162/166	163/165	239/245	242/258	175/185
837	Woodgrange Park	53	22/06/2001	194/194	162/170	155/163	239/245	256/264	173/173
838	Woodgrange Park	53	22/06/2001	212/212	162/174	155/167	245/245	254/254	175/179
839	Woodgrange Park	53	22/06/2001	208/216	170/174	155/167	239/239	248/250	/
840	Woodgrange Park	53	22/06/2001	206/206	170/170	163/163	239/245	246/260	173/179
841	St. James Lane	54	22/06/2001	196/206	166/170	155/171	245/245	256/258	/
842	St. James Lane	54	22/06/2001	192/216	168/170	155/165	239/239	246/256	/
843	St. James Lane	54	22/06/2001	196/200	170/170	163/163	239/239	252/252	181/181
844	St. James Lane	54	22/06/2001	182/182	162/162	155/155	245/245	252/254	179/181
845	St. James Lane	54	22/06/2001	198/208	170/170	155/163	239/239	242/246	/
846	St. James Lane	54	22/06/2001	182/182	162/162	155/155	245/245	256/256	181/181
847	St. James Lane	54	22/06/2001	182/196	170/170	155/167	239/243	246/248	/
848	St. James Lane	54	22/06/2001	188/196	168/170	157/165	239/245	/	179/181
849	St. James Lane	54	22/06/2001	182/182	162/170	155/155	239/239	246/260	173/179
850	St. James Lane	54	22/06/2001	194/194	170/174	155/167	239/245	258/258	179/179
851	Beddington Park	55	25/06/2001	210/210	162/166	155/165	239/245	254/254	175/175
852	Beddington Park	55	25/06/2001	182/206	170/174	155/163	239/239	246/250	191/191
853	Beddington Park	55	25/06/2001	196/208	170/182	165/165	247/255	242/250	/
854	Beddington Park	55	25/06/2001	182/182	162/170	155/157	239/245	/	173/175
855	Beddington Park	55	25/06/2001	182/198	170/174	/	239/245	254/258	189/189
856	Beddington Park	55	25/06/2001	196/198	174/174	155/163	239/239	246/256	179/179
857	Beddington Park	55	25/06/2001	182/198	174/174	155/167	239/245	256/256	177/179
858	Beddington Park	55	25/06/2001	198/200	162/168	157/163	239/245	256/258	179/181
859	Beddington Park	55	25/06/2001	210/210	162/162	155/165	239/239	254/254	175/175
860	Beddington Park	55	25/06/2001	208/208	170/170	155/165	243/245	246/246	173/175
871	Grove Park	56	26/06/2001	/	162/166	155/163	239/245	258/258	173/173
872	Grove Park	56	26/06/2001	188/196	170/170	157/157	245/245	250/250	175/179
873	Grove Park	56	26/06/2001	206/208	162/170	155/163	239/245	256/258	175/187
874	Grove Park	56	26/06/2001	182/208	174/174	155/159	245/245	/	177/185
875	Grove Park	56	26/06/2001	182/208	170/170	165/165	239/239	246/254	/

876	Grove Park	56	26/06/2001	188/196	170/170	157/157	239/245	246/250	175/179
877	Grove Park	56	26/06/2001	208/216	160/160	155/155	239/245	/	173/173
878	Grove Park	56	26/06/2001	196/206	160/160	155/163	243/245	246/254	/
879	Grove Park	56	26/06/2001	206/208	162/170	155/163	239/245	256/256	175/187
880	Grove Park	56	26/06/2001	182/188	166/172	155/157	239/247	250/256	181/189

Appendix E

Final genotypes used in analyses for *B. pascuorum*. A total of 458 workers were typed at an average of 5.8 (range 3-6) loci.

Sample ID	Sample Site	Patch	Date Collected	Locus B96	Locus B124	Locus B126	Locus B131	Locus B132	Locus B118
151	Barnes Common	1	03/07/2001	225/225	255/257	126/126	133/135	152/156	214/216
152	Barnes Common	1	03/07/2001	221/225	257/257	126/126	135/139	154/156	214/216
153	Barnes Common	1	03/07/2001	215/223	255/257	126/132	133/139	/	222/224
154	Barnes Common	1	03/07/2001	221/225	253/257	126/128	131/137	146/156	216/222
155	Barnes Common	1	03/07/2001	215/215	255/259	126/126	133/139	152/152	216/216
156	Barnes Common	1	03/07/2001	221/229	253/255	126/126	133/135	154/156	/
157	Barnes Common	1	03/07/2001	221/225	255/257	126/128	137/137	156/156	/
158	Barnes Common	1	03/07/2001	225/225	255/257	126/126	131/131	146/146	/
159	Barnes Common	1	03/07/2001	221/225	255/257	126/128	131/131	152/160	218/218
160	Barnes Common	1	03/07/2001	221/225	255/257	126/126	131/137	146/146	214/216
161	Barnes Common	2	03/07/2001	225/225	249/253	126/126	133/137	146/152	216/222
162	Barnes Common	2	03/07/2001	221/225	249/257	126/126	135/137	146/152	218/224
163	Barnes Common	2	03/07/2001	221/223	253/255	126/126	123/133	146/146	216/216
164	Barnes Common	2	03/07/2001	215/215	255/257	126/128	137/137	146/150	216/218
165	Barnes Common	2	03/07/2001	223/225	249/253	126/126	131/133	158/158	214/220
166	Barnes Common	2	03/07/2001	221/225	257/257	126/130	131/131	152/152	218/218
167	Barnes Common	2	03/07/2001	215/221	255/255	130/132	133/139	146/158	218/218
168	Barnes Common	2	03/07/2001	221/225	253/255	126/126	131/133	152/156	216/218
169	Barnes Common	2	03/07/2001	221/225	255/255	126/126	133/135	152/156	214/216
170	Barnes Common	2	03/07/2001	221/223	257/257	126/128	135/139	152/154	220/222
171	Barnes Common	3	03/07/2001	219/221	257/257	126/126	131/139	146/152	/
172	Barnes Common	3	03/07/2001	215/225	257/257	126/126	133/133	150/152	/
173	Barnes Common	3	03/07/2001	221/225	251/255	126/126	131/133	146/154	/
174	Barnes Common	3	03/07/2001	215/225	257/257	128/132	133/139	152/152	/
175	Barnes Common	3	03/07/2001	225/231	251/257	128/130	123/133	156/160	/
176	Barnes Common	3	03/07/2001	221/221	257/257	126/126	133/137	152/154	218/218
177	Barnes Common	3	03/07/2001	223/225	257/261	128/128	135/139	158/158	/
178	Barnes Common	3	03/07/2001	221/225	259/261	126/126	133/133	152/152	/
179	Barnes Common	3	03/07/2001	215/221	255/257	128/128	133/137	152/152	/
180	Barnes Common	3	03/07/2001	221/225	255/255	126/126	133/139	148/152	214/224
181	Barnes Common	4	03/07/2001	221/227	257/261	126/130	129/133	152/152	/
182	Barnes Common	4	03/07/2001	221/221	255/261	126/126	129/133	150/156	/
183	Barnes Common	4	03/07/2001	221/227	249/253	126/126	133/137	146/158	/
184	Barnes Common	4	03/07/2001	223/225	255/257	126/126	133/137	152/154	214/222
185	Barnes Common	4	03/07/2001	221/225	257/259	130/132	125/125	146/156	216/216
186	Barnes Common	4	03/07/2001	221/221	251/257	126/132	123/133	152/152	/
187	Barnes Common	4	03/07/2001	221/221	255/259	128/132	131/133	152/152	220/224
188	Barnes Common	4	03/07/2001	221/225	253/253	126/126	133/135	146/152	214/222
189	Barnes Common	4	03/07/2001	221/223	255/259	128/132	131/133	142/142	220/224

190	Barnes Common	4	03/07/2001	221/225	253/255	126/126	131/133	152/156	216/218
191	Barnes Common	5	03/07/2001	221/223	255/257	126/128	131/133	152/152	220/222
192	Barnes Common	5	03/07/2001	219/221	249/255	126/126	131/133	152/156	216/220
193	Barnes Common	5	03/07/2001	221/223	253/253	128/132	133/133	152/152	224/224
194	Barnes Common	5	03/07/2001	221/223	255/263	126/130	131/139	150/156	214/216
195	Barnes Common	5	03/07/2001	221/225	249/253	126/130	131/139	150/152	214/224
196	Barnes Common	5	03/07/2001	221/225	255/257	126/126	125/131	146/152	212/220
197	Barnes Common	5	03/07/2001	215/223	253/257	128/132	131/133	152/152	220/224
198	Barnes Common	5	03/07/2001	225/225	253/253	126/130	131/133	152/152	216/220
199	Barnes Common	5	03/07/2001	221/225	251/253	126/126	125/133	146/152	218/222
200	Barnes Common	5	27/06/2001	221/225	253/257	126/128	131/139	152/156	220/226
201	Barnes Common	6	27/06/2001	223/225	251/259	126/126	131/131	150/156	/
202	Barnes Common	6	27/06/2001	223/225	255/257	126/126	133/135	146/146	/
203	Barnes Common	6	27/06/2001	223/225	255/257	126/126	133/137	152/154	214/222
204	Barnes Common	6	27/06/2001	215/225	255/255	126/130	123/133	152/156	/
205	Barnes Common	6	27/06/2001	223/225	255/257	126/126	123/129	154/160	220/222
206	Barnes Common	6	27/06/2001	219/221	253/255	130/132	133/133	146/146	/
207	Barnes Common	6	27/06/2001	215/221	253/255	130/132	/	146/152	216/220
208	Barnes Common	6	27/06/2001	225/225	249/255	126/130	123/133	152/156	/
209	Barnes Common	6	27/06/2001	223/225	253/253	126/130	123/127	146/152	/
210	Barnes Common	6	27/06/2001	223/225	255/257	126/126	133/137	152/154	214/222
211	Barnes Common	7	27/06/2001	221/223	253/253	126/126	123/123	146/146	216/216
212	Barnes Common	7	27/06/2001	223/225	255/257	126/126	133/137	152/154	214/222
213	Barnes Common	7	27/06/2001	221/225	255/255	124/126	133/133	146/152	216/218
214	Barnes Common	7	27/06/2001	215/225	245/255	126/132	131/131	152/160	214/216
215	Barnes Common	7	27/06/2001	221/225	253/255	126/130	131/131	150/152	/
216	Barnes Common	7	27/06/2001	221/227	253/253	126/132	133/137	152/152	216/218
217	Barnes Common	7	27/06/2001	215/221	255/255	126/126	133/137	154/158	220/236
218	Barnes Common	7	27/06/2001	221/225	255/255	126/130	123/131	152/154	222/222
219	Barnes Common	7	27/06/2001	221/225	255/255	124/126	131/133	146/152	216/218
220	Barnes Common	7	27/06/2001	221/231	241/255	126/126	137/147	152/152	/
221	Barnes Common	8	27/06/2001	221/231	253/257	126/132	131/131	150/152	224/226
222	Barnes Common	8	27/06/2001	221/225	253/253	126/126	129/139	154/154	218/218
223	Barnes Common	8	27/06/2001	221/225	251/257	126/132	123/133	152/152	/
224	Barnes Common	8	27/06/2001	223/225	241/249	126/126	125/133	158/158	216/216
225	Barnes Common	8	27/06/2001	215/221	255/255	126/126	133/135	154/158	226/226
226	Barnes Common	8	27/06/2001	215/221	255/255	126/126	133/135	154/158	226/226
227	Barnes Common	8	27/06/2001	221/221	255/257	126/128	131/139	146/150	/
228	Barnes Common	8	27/06/2001	223/225	255/257	126/126	133/133	152/154	214/222
229	Barnes Common	8	27/06/2001	221/221	253/257	126/130	133/137	154/156	218/218
230	Barnes Common	8	27/06/2001	221/225	249/255	126/126	129/137	146/152	216/222
231	Barnes Common	9	27/06/2001	221/227	251/253	126/128	129/129	146/150	/
232	Barnes Common	9	27/06/2001	221/223	253/259	126/126	133/133	150/152	214/216
233	Barnes Common	9	27/06/2001	215/225	255/255	126/126	133/137	152/152	/

234	Barnes Common	9	27/06/2001	227/231	249/255	126/128	133/133	152/160	224/224
235	Barnes Common	9	27/06/2001	229/229	251/253	128/130	133/135	152/154	/
236	Barnes Common	9	27/06/2001	221/223	255/263	126/126	135/139	150/152	214/216
237	Barnes Common	9	27/06/2001	221/225	253/257	126/126	135/139	154/156	218/218
238	Barnes Common	9	27/06/2001	221/225	245/249	126/126	135/139	154/156	/
239	Barnes Common	9	27/06/2001	223/225	255/255	126/132	129/131	152/152	/
240	Barnes Common	9	27/06/2001	225/225	255/255	126/130	133/133	146/146	/
241	Barnes Common	10	27/06/2001	215/221	255/255	126/130	133/147	150/156	226/228
242	Barnes Common	10	27/06/2001	221/221	255/257	126/126	129/131	150/152	/
243	Barnes Common	10	27/06/2001	219/221	251/257	126/126	129/131	152/152	/
244	Barnes Common	10	27/06/2001	221/225	257/259	126/132	131/137	152/152	/
245	Barnes Common	10	27/06/2001	221/227	255/257	126/126	131/131	152/152	/
246	Barnes Common	10	27/06/2001	221/225	253/253	126/130	123/123	152/154	222/222
247	Barnes Common	10	27/06/2001	221/231	257/257	126/126	133/139	146/152	/
248	Barnes Common	10	27/06/2001	223/225	255/257	126/130	129/137	156/156	220/220
249	Barnes Common	10	27/06/2001	221/225	253/253	126/130	123/127	152/152	/
250	Barnes Common	10	27/06/2001	215/225	255/259	126/132	123/127	152/160	214/216
301	Nunhead Cemetery	11	10/07/2001	221/227	257/259	126/126	123/133	152/152	214/214
302	Nunhead Cemetery	11	10/07/2001	219/221	255/261	126/126	131/133	152/154	216/220
303	Nunhead Cemetery	11	10/07/2001	219/221	259/261	126/126	123/135	152/154	222/222
304	Nunhead Cemetery	11	10/07/2001	221/225	251/259	126/126	123/125	154/156	216/224
305	Nunhead Cemetery	11	10/07/2001	225/225	257/257	126/128	131/137	/	222/224
306	Nunhead Cemetery	11	10/07/2001	225/225	257/257	126/132	135/135	150/150	216/216
307	Nunhead Cemetery	11	10/07/2001	221/221	257/261	132/132	131/133	150/152	214/216
308	Nunhead Cemetery	11	10/07/2001	221/225	251/259	126/128	133/135	150/156	220/234
309	Nunhead Cemetery	11	10/07/2001	221/223	251/257	126/126	131/131	150/152	214/220
310	Nunhead Cemetery	11	10/07/2001	221/221	257/259	126/126	123/133	152/152	222/224
311	Nunhead Cemetery	12	10/07/2001	221/225	251/261	126/126	123/135	154/156	216/224
312	Nunhead Cemetery	12	10/07/2001	225/225	257/259	126/128	131/137	146/152	214/220
313	Nunhead Cemetery	12	10/07/2001	221/221	251/257	128/128	133/139	142/152	222/226
314	Nunhead Cemetery	12	10/07/2001	221/225	257/257	126/126	133/137	154/156	214/224
315	Nunhead Cemetery	12	10/07/2001	221/221	249/251	128/134	131/133	154/160	216/218
316	Nunhead Cemetery	12	10/07/2001	215/225	255/257	126/126	133/133	/	216/216
317	Nunhead Cemetery	12	10/07/2001	221/229	255/259	126/126	131/137	152/154	224/224
318	Nunhead Cemetery	12	10/07/2001	221/223	251/257	126/126	131/131	150/152	214/220
319	Nunhead Cemetery	12	10/07/2001	215/221	257/257	126/126	133/135	150/156	220/234
320	Nunhead Cemetery	12	10/07/2001	221/225	251/257	126/128	133/135	156/156	220/222
321	Nunhead Cemetery	13	10/07/2001	225/227	/	126/128	131/133	150/152	226/226
322	Nunhead Cemetery	13	10/07/2001	221/229	255/257	126/126	125/125	146/152	220/226
323	Nunhead Cemetery	13	10/07/2001	225/225	253/257	126/126	131/133	154/160	220/222
324	Nunhead Cemetery	13	10/07/2001	219/225	251/261	126/126	133/137	150/160	/
325	Nunhead Cemetery	13	10/07/2001	221/225	249/255	126/126	129/137	156/160	216/216
326	Nunhead Cemetery	13	10/07/2001	221/221	255/257	126/126	123/133	152/152	216/218
327	Nunhead Cemetery	13	10/07/2001	221/227	257/259	126/126	131/131	146/150	218/222
328	Nunhead Cemetery	13	10/07/2001	225/227	255/257	126/126	133/133	138/142	222/224
329	Nunhead Cemetery	13	10/07/2001	225/225	253/257	126/126	133/133	152/160	220/224
330	Nunhead Cemetery	13	10/07/2001	221/225	249/253	126/126	123/133	150/152	216/216
331	Nunhead Cemetery	14	10/07/2001	223/229	257/257	126/126	133/139	150/150	218/220

332	Nunhead Cemetery	14	10/07/2001	221/221	255/259	126/126	131/133	150/150	216/216
333	Nunhead Cemetery	14	10/07/2001	223/229	255/255	126/130	131/137	150/152	220/222
334	Nunhead Cemetery	14	10/07/2001	223/225	/	126/132	123/123	152/154	216/220
335	Nunhead Cemetery	14	10/07/2001	225/225	255/257	126/126	129/133	152/158	/
336	Nunhead Cemetery	14	10/07/2001	221/225	251/259	126/132	131/133	142/152	216/222
337	Nunhead Cemetery	14	10/07/2001	221/221	/	128/134	129/133	150/152	216/222
338	Nunhead Cemetery	14	10/07/2001	221/225	259/259	126/128	123/133	142/152	214/220
339	Nunhead Cemetery	14	10/07/2001	221/221	253/257	126/126	131/133	152/152	/
340	Nunhead Cemetery	14	10/07/2001	221/221	255/259	126/132	131/147	142/152	/
341	Nunhead Cemetery	15	10/07/2001	219/221	255/255	126/128	123/135	152/154	222/222
342	Nunhead Cemetery	15	10/07/2001	221/223	251/253	126/126	133/137	152/154	214/226
343	Nunhead Cemetery	15	10/07/2001	225/225	249/253	126/126	133/137	150/154	216/222
344	Nunhead Cemetery	15	10/07/2001	221/221	257/259	126/126	121/131	152/154	220/220
345	Nunhead Cemetery	15	10/07/2001	221/225	/	126/128	123/129	152/154	216/220
346	Nunhead Cemetery	15	10/07/2001	221/225	249/255	126/126	123/129	152/156	216/218
347	Nunhead Cemetery	15	10/07/2001	219/221	255/257	126/130	131/137	154/158	218/222
348	Nunhead Cemetery	15	10/07/2001	221/225	255/259	126/130	129/131	152/156	212/218
349	Nunhead Cemetery	15	10/07/2001	221/227	257/259	126/130	121/131	156/156	216/220
350	Nunhead Cemetery	15	10/07/2001	221/227	253/253	126/126	123/133	152/152	218/222
351	Nunhead Cemetery	16	12/07/2001	221/223	/	134/134	125/133	/	224/230
352	Nunhead Cemetery	16	12/07/2001	221/225	257/257	126/132	135/137	150/162	216/222
353	Nunhead Cemetery	16	12/07/2001	221/225	251/257	126/134	135/139	154/156	218/224
354	Nunhead Cemetery	16	12/07/2001	221/223	/	126/126	133/133	152/160	216/222
355	Nunhead Cemetery	16	12/07/2001	221/223	255/257	126/126	131/135	152/156	216/222
356	Nunhead Cemetery	16	12/07/2001	221/223	251/257	126/134	133/133	152/156	/
357	Nunhead Cemetery	16	12/07/2001	221/225	251/259	126/128	133/135	150/156	220/234
358	Nunhead Cemetery	16	12/07/2001	221/225	255/257	126/130	131/137	154/158	218/220
359	Nunhead Cemetery	16	12/07/2001	225/227	255/255	128/132	133/135	142/158	220/222
360	Nunhead Cemetery	16	12/07/2001	221/223	/	126/128	133/133	152/160	216/222
361	Nunhead Cemetery	17	12/07/2001	221/223	249/259	126/126	133/133	152/156	218/224
362	Nunhead Cemetery	17	12/07/2001	225/229	253/257	128/132	131/131	152/160	216/224
363	Nunhead Cemetery	17	12/07/2001	225/227	255/255	128/132	133/133	154/158	220/222
364	Nunhead Cemetery	17	12/07/2001	225/225	259/259	126/126	123/133	150/154	218/224
365	Nunhead Cemetery	17	12/07/2001	225/225	253/257	126/128	133/133	150/150	216/218
366	Nunhead Cemetery	17	12/07/2001	221/225	251/261	126/126	123/135	154/156	216/224
367	Nunhead Cemetery	17	12/07/2001	227/231	255/255	126/126	131/133	142/150	216/216
368	Nunhead Cemetery	17	12/07/2001	227/231	255/257	126/126	131/133	152/160	216/218
369	Nunhead Cemetery	17	12/07/2001	221/225	251/259	126/126	123/125	154/156	216/224
370	Nunhead Cemetery	17	12/07/2001	225/225	257/259	126/128	131/137	146/152	214/220
371	Nunhead Cemetery	18	12/07/2001	225/227	255/257	126/126	133/133	138/142	220/224
372	Nunhead Cemetery	18	12/07/2001	225/225	251/255	126/126	131/133	142/152	216/224
373	Nunhead Cemetery	18	12/07/2001	221/221	255/257	126/132	123/131	142/142	214/230
374	Nunhead Cemetery	18	12/07/2001	221/227	251/257	126/128	131/133	152/152	216/218
375	Nunhead Cemetery	18	12/07/2001	221/225	257/259	/	133/141	152/154	218/218
376	Nunhead Cemetery	18	12/07/2001	225/225	253/257	126/128	133/133	150/150	216/218
377	Nunhead Cemetery	18	12/07/2001	221/225	255/257	126/126	131/137	154/156	214/224
378	Nunhead Cemetery	18	12/07/2001	225/227	249/255	126/128	133/133	150/154	216/222
379	Nunhead Cemetery	18	12/07/2001	225/225	249/255	126/132	133/133	146/146	214/226
380	Nunhead Cemetery	18	12/07/2001	225/227	249/257	126/130	129/133	150/152	222/230
381	Nunhead Cemetery	19	12/07/2001	221/223	251/253	126/126	133/137	152/154	214/226

382	Nunhead Cemetery	19	12/07/2001	225/225	257/259	126/128	131/137	146/152	214/220
383	Nunhead Cemetery	19	12/07/2001	219/221	255/261	126/126	131/133	152/154	216/220
384	Nunhead Cemetery	19	12/07/2001	221/227	257/259	126/126	123/133	152/152	214/214
385	Nunhead Cemetery	19	12/07/2001	223/229	257/257	126/126	133/139	150/150	218/220
386	Nunhead Cemetery	19	12/07/2001	225/225	257/259	126/128	131/137	146/152	214/220
387	Nunhead Cemetery	19	12/07/2001	221/221	251/257	128/128	133/139	142/152	222/226
388	Nunhead Cemetery	19	12/07/2001	221/221	257/259	126/126	123/133	152/152	222/224
389	Nunhead Cemetery	19	12/07/2001	221/225	257/257	126/126	133/137	154/156	214/224
390	Nunhead Cemetery	19	12/07/2001	221/221	249/251	128/134	131/133	154/160	216/218
391	Nunhead Cemetery	20	12/07/2001	221/221	255/259	126/126	131/133	150/150	216/216
392	Nunhead Cemetery	20	12/07/2001	225/225	249/253	126/126	133/137	150/154	216/222
393	Nunhead Cemetery	20	12/07/2001	221/221	251/257	128/128	133/139	142/152	222/226
394	Nunhead Cemetery	20	12/07/2001	221/221	251/257	128/128	133/139	142/152	222/226
395	Nunhead Cemetery	20	12/07/2001	221/225	249/253	126/126	123/133	150/152	216/216
396	Nunhead Cemetery	20	12/07/2001	219/221	259/261	126/126	123/135	152/154	222/222
397	Nunhead Cemetery	20	12/07/2001	221/223	251/257	126/126	131/131	150/152	214/220
398	Nunhead Cemetery	20	12/07/2001	221/221	257/259	126/126	121/131	152/154	220/220
399	Nunhead Cemetery	20	12/07/2001	225/225	253/257	126/126	133/133	152/160	220/224
400	Nunhead Cemetery	20	12/07/2001	221/225	251/261	126/126	123/135	154/156	216/224
401	Regent's Park	21	20/07/2001	221/225	251/257	126/126	127/129	150/152	/
402	Regent's Park	21	20/07/2001	221/227	251/255	126/132	133/133	152/152	/
403	Regent's Park	21	20/07/2001	221/221	251/257	126/126	131/131	146/152	/
404	Regent's Park	21	20/07/2001	221/223	251/261	126/132	131/133	150/152	/
405	Regent's Park	21	20/07/2001	221/221	257/257	126/126	133/135	152/152	216/220
406	Regent's Park	21	20/07/2001	221/225	253/255	126/128	123/137	152/156	216/220
407	Regent's Park	21	20/07/2001	221/225	253/259	126/126	133/137	150/152	214/218
408	Regent's Park	21	20/07/2001	215/221	249/253	130/134	133/139	146/156	214/214
409	Regent's Park	21	20/07/2001	221/227	253/255	126/128	131/139	146/150	224/224
410	Regent's Park	21	20/07/2001	221/221	257/259	126/126	133/137	146/152	214/218
411	Regent's Park	22	20/07/2001	221/221	249/253	126/132	131/131	146/152	220/236
412	Regent's Park	22	20/07/2001	221/225	253/253	126/126	133/135	150/156	216/220
413	Regent's Park	22	20/07/2001	215/221	249/253	130/134	133/139	146/156	214/214
414	Regent's Park	22	20/07/2001	225/227	245/249	126/126	133/133	150/156	216/220
415	Regent's Park	22	20/07/2001	225/229	255/255	126/128	135/139	152/162	/
416	Regent's Park	22	20/07/2001	221/225	249/259	126/130	131/135	150/152	/
417	Regent's Park	22	20/07/2001	221/221	249/253	126/132	131/131	154/154	/
418	Regent's Park	22	20/07/2001	221/227	253/255	126/128	131/139	146/150	224/224
419	Regent's Park	22	20/07/2001	221/221	249/253	126/130	139/139	152/152	224/226
420	Regent's Park	22	20/07/2001	221/223	249/255	130/132	129/137	152/152	216/220
421	Regent's Park	23	20/07/2001	221/227	255/255	126/132	131/141	146/146	216/222
422	Regent's Park	23	20/07/2001	221/221	255/259	126/128	129/139	152/154	214/224
423	Regent's Park	23	20/07/2001	225/225	253/253	126/126	133/135	150/156	216/232
424	Regent's Park	23	20/07/2001	221/221	249/257	126/132	125/125	142/154	216/222
425	Regent's Park	23	20/07/2001	221/225	253/259	126/128	133/135	152/158	216/220
426	Regent's Park	23	20/07/2001	221/221	255/255	126/126	139/139	156/162	214/214
427	Regent's Park	23	20/07/2001	221/225	251/257	128/128	131/137	142/154	220/222

428	Regent's Park	23	20/07/2001	221/227	255/255	126/126	131/133	146/146	216/222
429	Regent's Park	23	20/07/2001	221/221	257/259	126/126	133/137	146/152	214/218
430	Regent's Park	23	20/07/2001	221/225	253/255	126/128	123/137	152/156	216/220
431	Regent's Park	24	20/07/2001	215/221	253/255	128/132	129/133	146/160	222/224
432	Regent's Park	24	20/07/2001	221/225	253/259	126/126	133/137	150/152	214/218
433	Regent's Park	24	20/07/2001	221/221	249/257	130/132	131/133	152/158	216/224
434	Regent's Park	24	20/07/2001	221/225	253/259	126/130	131/137	152/156	218/222
435	Regent's Park	24	20/07/2001	221/223	257/257	126/126	123/123	154/162	214/218
436	Regent's Park	24	20/07/2001	221/227	253/259	126/126	129/133	146/150	222/224
437	Regent's Park	24	20/07/2001	221/221	257/259	126/126	131/133	146/162	216/222
438	Regent's Park	24	20/07/2001	223/227	253/257	116/116	123/123	150/150	/
439	Regent's Park	24	20/07/2001	215/225	255/255	126/130	131/131	150/152	218/222
440	Regent's Park	25	20/07/2001	221/225	257/259	126/130	133/137	146/152	216/218
441	Regent's Park	25	20/07/2001	223/227	259/261	126/126	133/139	146/150	224/226
442	Regent's Park	25	20/07/2001	221/221	257/259	128/132	133/137	152/152	222/224
443	Regent's Park	25	20/07/2001	221/221	257/257	126/128	139/141	158/158	/
444	Regent's Park	25	20/07/2001	221/225	251/257	126/126	129/129	150/150	/
445	Regent's Park	25	20/07/2001	221/221	255/255	126/126	123/129	150/154	216/224
446	Regent's Park	25	20/07/2001	225/225	251/259	126/126	127/131	152/152	216/220
447	Regent's Park	25	20/07/2001	221/225	259/259	126/130	133/137	146/152	216/218
448	Regent's Park	25	20/07/2001	221/221	255/255	132/136	133/137	150/150	216/220
449	Regent's Park	25	20/07/2001	221/225	251/257	128/128	131/131	142/154	220/222
450	Regent's Park	25	20/07/2001	221/221	257/257	126/128	129/133	150/154	216/224
451	Regent's Park	26	20/07/2001	215/225	255/255	126/128	131/131	152/152	218/222
452	Regent's Park	26	20/07/2001	221/221	251/257	126/126	131/133	152/152	224/234
453	Regent's Park	26	20/07/2001	225/225	255/255	128/128	133/141	156/158	216/216
454	Regent's Park	26	20/07/2001	221/227	251/257	126/126	131/133	142/150	216/224
455	Regent's Park	26	20/07/2001	221/223	255/257	126/126	123/123	142/150	220/228
456	Regent's Park	26	20/07/2001	221/223	255/257	126/126	123/133	142/150	/
457	Regent's Park	26	20/07/2001	221/225	249/257	126/130	131/137	142/152	222/224
458	Regent's Park	26	20/07/2001	221/221	255/255	126/126	123/129	150/154	216/224
459	Regent's Park	26	20/07/2001	223/223	253/257	126/126	133/135	142/142	216/232
460	Regent's Park	26	20/07/2001	223/227	255/257	126/128	131/137	142/152	220/222
461	Regent's Park	27	20/07/2001	221/225	255/257	128/128	133/139	142/152	222/224
462	Regent's Park	27	20/07/2001	215/221	257/259	/	131/141	142/142	224/226
463	Regent's Park	27	20/07/2001	221/225	251/257	128/128	129/131	150/152	/
464	Regent's Park	27	20/07/2001	221/227	257/257	126/126	139/141	142/158	/
465	Regent's Park	27	20/07/2001	215/221	287/287	126/126	133/135	154/154	222/224
466	Regent's Park	27	20/07/2001	223/227	257/257	116/116	123/123	150/150	/
467	Regent's Park	27	20/07/2001	221/223	253/253	126/128	133/137	150/152	222/224
468	Regent's Park	27	20/07/2001	221/223	255/257	126/128	125/131	142/152	220/222
469	Regent's Park	27	20/07/2001	221/223	257/257	116/116	123/133	150/150	/
470	Regent's Park	27	20/07/2001	215/225	253/259	126/128	125/129	142/150	220/220
471	Regent's Park	28	20/07/2001	221/227	249/257	126/126	129/133	154/158	/

472	Regent's Park	28	20/07/2001	221/223	249/255	130/132	129/137	152/152	216/220
473	Regent's Park	28	20/07/2001	221/221	249/257	126/126	131/133	152/152	/
474	Regent's Park	28	20/07/2001	221/227	255/257	126/128	131/137	142/152	220/222
475	Regent's Park	28	20/07/2001	221/227	255/255	126/132	139/141	146/146	/
476	Regent's Park	28	20/07/2001	221/227	249/257	126/132	131/137	152/152	/
477	Regent's Park	28	20/07/2001	221/221	249/257	126/132	131/131	152/158	/
478	Regent's Park	28	20/07/2001	221/221	251/255	126/126	123/133	152/152	/
479	Regent's Park	28	20/07/2001	221/227	251/257	126/126	131/133	142/150	220/222
480	Regent's Park	28	20/07/2001	221/225	253/255	126/128	123/123	152/156	216/220
481	Regent's Park	29	20/07/2001	223/225	257/259	128/128	131/141	142/154	220/222
482	Regent's Park	29	20/07/2001	215/221	287/287	126/126	133/135	154/154	222/224
483	Regent's Park	29	20/07/2001	215/225	249/249	140/140	133/141	156/158	/
484	Regent's Park	29	20/07/2001	221/225	257/257	126/126	133/137	142/152	214/214
485	Regent's Park	29	20/07/2001	221/225	257/259	126/126	133/137	142/152	214/216
486	Regent's Park	29	20/07/2001	221/227	251/255	126/126	133/133	152/158	/
487	Regent's Park	29	20/07/2001	221/223	255/259	126/128	133/137	150/152	222/224
488	Regent's Park	29	20/07/2001	215/225	253/253	126/126	131/133	146/162	218/222
489	Regent's Park	29	20/07/2001	221/225	245/255	126/128	135/137	152/156	216/220
490	Regent's Park	29	20/07/2001	215/225	255/255	126/128	131/133	152/152	218/222
491	Regent's Park	30	20/07/2001	221/221	251/255	126/126	139/147	156/162	214/214
492	Regent's Park	30	20/07/2001	221/221	255/261	132/136	133/137	150/150	216/220
493	Regent's Park	30	20/07/2001	221/227	251/257	126/126	133/133	142/150	/
494	Regent's Park	30	20/07/2001	221/227	251/257	126/126	133/133	142/150	/
495	Regent's Park	30	20/07/2001	221/223	253/253	126/128	133/137	150/152	222/224
496	Regent's Park	30	20/07/2001	221/223	257/257	126/126	123/123	154/162	214/218
497	Regent's Park	30	20/07/2001	221/223	249/255	130/132	129/137	152/152	216/220
498	Regent's Park	30	20/07/2001	221/225	243/251	128/128	131/137	142/154	220/222
499	Regent's Park	30	20/07/2001	221/227	251/257	132/132	131/133	142/150	216/224
500	Regent's Park	30	20/07/2001	215/221	253/255	128/132	129/133	146/160	222/224
601	Millenium Village	31	27/07/2001	219/221	253/257	126/126	133/139	152/156	212/226
602	Millenium Village	31	27/07/2001	221/221	253/263	126/126	133/133	150/160	214/216
603	Millenium Village	31	27/07/2001	221/227	249/255	126/126	123/131	146/160	214/218
604	Millenium Village	31	27/07/2001	225/225	255/257	126/128	123/133	146/152	214/222
605	Millenium Village	31	27/07/2001	221/225	253/253	126/128	141/151	150/152	214/234
606	Millenium Village	31	27/07/2001	219/221	253/257	126/126	131/139	150/152	212/226
607	Millenium Village	31	27/07/2001	219/223	263/263	128/132	143/149	146/154	220/222
608	Millenium Village	31	27/07/2001	221/225	255/255	126/126	133/137	146/154	216/218
609	Millenium Village	31	27/07/2001	223/225	255/255	126/126	131/131	150/152	218/218
610	Millenium Village	31	27/07/2001	225/225	253/257	126/126	123/131	160/160	218/218
611	Millenium Village	32	27/07/2001	219/227	255/255	126/128	121/131	152/152	214/214
612	Millenium Village	32	27/07/2001	225/225	253/257	126/126	123/131	152/154	218/234
613	Millenium Village	32	27/07/2001	221/225	253/253	126/126	133/139	150/152	214/234
614	Millenium Village	32	27/07/2001	223/225	255/255	126/126	131/131	150/152	218/218
615	Millenium Village	32	27/07/2001	221/225	249/255	126/132	123/131	146/160	214/218
616	Millenium Village	32	27/07/2001	221/223	253/253	128/132	133/137	150/154	214/214
617	Millenium Village	32	27/07/2001	221/225	255/255	126/132	133/133	/	212/212

618	Millenium Village	32	27/07/2001	219/223	263/263	128/132	143/149	146/154	220/222
619	Millenium Village	32	27/07/2001	221/221	255/255	126/126	121/121	146/152	216/220
620	Millenium Village	32	27/07/2001	221/225	261/261	126/126	121/131	146/152	216/218
621	Millenium Village	33	27/07/2001	221/225	255/255	126/126	133/137	146/154	216/218
622	Millenium Village	33	27/07/2001	223/223	249/249	126/126	133/137	146/156	214/214
623	Millenium Village	33	27/07/2001	221/225	253/253	126/126	133/139	150/152	214/234
624	Millenium Village	33	27/07/2001	221/225	255/255	126/126	133/137	146/154	216/218
625	Millenium Village	33	27/07/2001	223/225	249/249	126/126	125/133	150/152	214/234
626	Millenium Village	33	27/07/2001	221/221	253/263	126/126	133/133	150/160	214/216
627	Millenium Village	33	27/07/2001	221/223	255/259	126/128	131/133	/	220/220
628	Millenium Village	33	27/07/2001	219/223	249/257	126/126	131/133	/	214/214
629	Millenium Village	33	27/07/2001	219/223	241/249	116/126	123/123	146/154	/
630	Millenium Village	33	27/07/2001	221/227	255/257	126/130	123/133	/	214/218
631	Millenium Village	34	27/07/2001	225/225	249/257	126/126	133/139	150/152	214/234
632	Millenium Village	34	27/07/2001	223/227	253/255	126/132	139/139	150/154	214/234
633	Millenium Village	34	27/07/2001	221/225	255/261	126/126	121/131	146/152	216/218
634	Millenium Village	34	27/07/2001	221/225	249/255	126/132	123/131	146/160	214/218
635	Millenium Village	34	27/07/2001	225/225	249/257	126/126	133/139	150/152	214/234
636	Millenium Village	34	27/07/2001	221/227	255/257	126/126	133/135	154/158	218/226
637	Millenium Village	34	27/07/2001	221/227	249/255	126/126	123/131	146/160	214/218
638	Millenium Village	34	27/07/2001	221/225	257/259	126/126	129/129	150/160	214/216
639	Millenium Village	34	27/07/2001	221/223	255/257	126/126	123/133	152/152	214/222
640	Millenium Village	34	27/07/2001	221/221	255/257	126/126	121/121	146/152	216/218
641	Millenium Village	35	27/07/2001	221/225	255/255	126/128	131/131	156/160	216/220
642	Millenium Village	35	27/07/2001	/	/	/	/	/	/
643	Millenium Village	35	27/07/2001	/	/	/	/	/	/
644	Millenium Village	35	27/07/2001	221/223	249/255	126/132	133/137	150/154	220/220
645	Millenium Village	35	27/07/2001	223/227	253/255	126/132	139/139	150/154	214/234
646	Millenium Village	35	27/07/2001	225/225	253/257	126/126	123/131	152/154	218/234
647	Millenium Village	35	27/07/2001	221/221	253/259	126/126	127/131	146/154	216/220
648	Millenium Village	35	27/07/2001	221/225	257/259	126/126	129/129	150/160	214/216
649	Millenium Village	35	27/07/2001	219/223	263/263	128/132	143/149	146/154	220/222
650	Millenium Village	35	27/07/2001	221/223	249/255	126/132	133/137	150/154	220/220
651	Millenium Village	36	30/07/2001	221/225	249/255	126/132	123/131	146/160	214/218
652	Millenium Village	36	30/07/2001	221/227	249/255	126/130	123/131	146/160	214/218
653	Millenium Village	36	30/07/2001	221/225	255/255	126/126	131/133	/	220/222
654	Millenium Village	36	30/07/2001	221/221	253/263	126/126	129/131	150/160	214/216
655	Millenium Village	36	30/07/2001	223/225	249/255	126/126	133/139	150/152	214/234
656	Millenium Village	36	30/07/2001	223/227	253/255	126/132	139/139	150/154	214/234
657	Millenium Village	36	30/07/2001	221/221	253/259	126/126	127/131	146/154	216/220
658	Millenium Village	36	30/07/2001	221/225	253/255	126/126	121/131	152/156	216/218
659	Millenium Village	36	30/07/2001	219/227	255/255	126/128	121/131	152/152	214/214
660	Millenium Village	36	30/07/2001	223/225	249/255	126/126	133/139	150/152	214/234
661	Millenium Village	37	30/07/2001	221/223	249/255	126/132	133/137	150/154	214/220
662	Millenium Village	37	30/07/2001	221/223	255/255	126/126	135/139	152/152	220/220
663	Millenium Village	37	30/07/2001	221/225	257/259	126/126	129/129	150/160	214/216
664	Millenium Village	37	30/07/2001	219/223	249/259	126/126	127/129	146/152	214/222
665	Millenium Village	37	30/07/2001	223/225	249/249	126/126	133/139	150/152	214/234
666	Millenium Village	37	30/07/2001	221/225	253/255	126/130	131/133	152/154	/
667	Millenium Village	37	30/07/2001	221/221	255/255	126/126	121/121	146/152	216/220

668	Millenium Village	37	30/07/2001	225/225	253/257	126/134	123/131	152/154	218/234
669	Millenium Village	37	30/07/2001	225/225	249/257	126/126	133/139	150/152	214/234
670	Millenium Village	37	30/07/2001	221/227	255/257	126/126	133/135	154/158	218/226
671	Millenium Village	38	30/07/2001	221/223	249/255	126/132	133/137	150/150	214/220
672	Millenium Village	38	30/07/2001	221/225	255/259	126/128	131/139	146/154	/
673	Millenium Village	38	30/07/2001	221/223	253/255	126/130	127/129	146/152	218/220
674	Millenium Village	38	30/07/2001	221/227	255/257	126/126	133/135	154/158	218/226
675	Millenium Village	38	30/07/2001	221/227	255/259	126/126	/	146/152	220/222
676	Millenium Village	38	30/07/2001	221/225	259/265	126/126	129/129	150/160	214/216
677	Millenium Village	38	30/07/2001	227/227	255/257	126/126	133/135	146/146	218/218
678	Millenium Village	38	30/07/2001	221/225	255/261	126/126	121/131	146/152	216/218
679	Millenium Village	38	30/07/2001	221/225	257/259	126/126	129/129	150/160	214/216
680	Millenium Village	38	30/07/2001	223/223	249/249	126/126	133/137	146/156	214/214
681	Millenium Village	39	30/07/2001	221/223	249/255	126/132	133/137	150/154	220/220
682	Millenium Village	39	30/07/2001	223/227	253/255	126/132	139/139	150/154	214/234
683	Millenium Village	39	30/07/2001	221/225	249/255	126/132	123/131	146/160	214/218
684	Millenium Village	39	30/07/2001	/	257/257	126/126	131/131	146/146	222/224
685	Millenium Village	39	30/07/2001	223/225	253/253	126/126	131/131	152/152	220/234
686	Millenium Village	39	30/07/2001	221/225	255/255	126/126	121/121	146/152	216/218
687	Millenium Village	39	30/07/2001	215/225	251/255	126/128	/	146/154	228/228
688	Millenium Village	39	30/07/2001	223/227	253/255	126/132	139/139	150/154	214/234
689	Millenium Village	39	30/07/2001	225/225	255/257	126/128	133/133	146/152	214/222
690	Millenium Village	39	30/07/2001	221/221	255/257	126/126	121/121	146/152	216/218
691	Millenium Village	40	30/07/2001	221/225	255/261	126/126	121/131	146/152	216/218
692	Millenium Village	40	30/07/2001	223/225	255/255	126/128	131/133	152/152	220/234
693	Millenium Village	40	30/07/2001	221/221	253/259	126/126	127/131	146/154	216/220
694	Millenium Village	40	30/07/2001	221/225	257/259	126/126	129/129	150/160	214/216
695	Millenium Village	40	30/07/2001	221/223	251/257	126/130	127/129	146/152	220/220
696	Millenium Village	40	30/07/2001	221/223	255/257	126/126	123/133	152/152	214/222
697	Millenium Village	40	30/07/2001	221/225	255/255	126/128	131/131	156/160	216/220
698	Millenium Village	40	30/07/2001	225/225	253/257	126/126	123/131	152/154	218/234
699	Millenium Village	40	30/07/2001	221/223	257/263	126/132	129/129	146/152	218/220
700	Millenium Village	40	30/07/2001	221/227	249/255	126/126	123/131	146/160	214/216
821	Woodgrange Park	41	22/06/2001	215/223	251/253	126/126	137/147	146/154	216/238
822	Woodgrange Park	41	22/06/2001	221/221	249/255	126/132	129/133	150/152	226/230
823	Woodgrange Park	41	22/06/2001	221/225	255/257	126/128	121/121	160/162	216/218
824	Woodgrange Park	41	22/06/2001	219/223	249/255	128/134	123/123	152/156	220/330
825	Woodgrange Park	41	22/06/2001	221/221	249/253	126/130	133/135	154/156	216/216
826	Woodgrange Park	41	22/06/2001	221/225	253/255	126/126	133/139	152/154	222/222
827	Woodgrange Park	41	22/06/2001	221/225	221/227	128/130	123/131	150/162	216/222
828	Woodgrange Park	41	22/06/2001	225/231	221/225	126/126	133/133	160/160	218/226
829	Woodgrange Park	41	22/06/2001	223/225	253/255	126/126	131/139	154/158	214/216
830	Woodgrange Park	41	22/06/2001	223/223	225/225	126/130	131/141	154/156	234/238
861	Beddington Park	42	25/06/2001	215/225	/	126/132	123/137	152/152	222/222
862	Beddington Park	42	25/06/2001	221/225	257/257	126/126	127/129	150/152	216/218
863	Beddington Park	42	25/06/2001	219/219	255/259	126/126	121/121	152/152	/
864	Beddington Park	42	25/06/2001	221/227	255/261	126/126	/	150/156	214/216
865	Beddington Park	42	25/06/2001	221/221	249/255	128/130	131/133	154/160	/
866	Beddington Park	42	25/06/2001	217/225	249/257	126/126	131/139	150/156	216/220
867	Beddington Park	42	25/06/2001	221/221	251/255	126/126	129/129	152/152	214/216

868	Beddington Park	42	25/06/2001	221/221	257/259	128/132	123/123	154/156	214/216
869	Beddington Park	42	25/06/2001	223/225	255/255	126/132	127/129	146/152	214/222
870	Beddington Park	42	25/06/2001	221/227	249/255	126/126	131/135	146/146	220/222
881	Grove Park	43	26/06/2001	223/225	253/257	126/126	123/133	152/158	216/220
882	Grove Park	43	26/06/2001	215/221	249/255	126/128	125/129	152/152	214/216
883	Grove Park	43	26/06/2001	221/223	253/259	126/126	131/133	154/156	214/220
884	Grove Park	43	26/06/2001	221/221	249/257	/	135/137	152/160	214/214
885	Grove Park	43	26/06/2001	221/221	255/257	126/128	133/141	160/160	216/236
886	Grove Park	43	26/06/2001	221/227	255/255	126/126	135/135	154/156	220/236
887	Grove Park	43	26/06/2001	221/221	249/259	126/126	123/137	146/146	/
888	Grove Park	43	26/06/2001	221/223	253/259	126/126	131/133	154/156	214/220
889	Grove Park	43	26/06/2001	221/225	253/253	126/126	131/131	146/152	214/222
890	Grove Park	43	26/06/2001	221/225	253/257	126/126	133/133	154/154	216/226
891	Hanwell	44	30/06/2001	221/221	249/249	126/128	133/139	154/156	214/220
892	Hanwell	44	30/06/2001	215/225	255/257	126/132	123/133	/	218/218
893	Hanwell	44	30/06/2001	221/223	249/249	126/128	137/141	/	214/216
894	Hanwell	44	30/06/2001	223/225	249/259	126/128	123/133	146/152	216/218
895	Hanwell	44	30/06/2001	221/223	255/261	126/134	131/135	152/152	222/226
896	Hanwell	44	30/06/2001	221/223	257/257	126/126	131/135	/	216/226
897	Hanwell	44	30/06/2001	221/225	249/251	128/134	139/139	142/152	222/226
898	Hanwell	44	30/06/2001	221/225	255/259	128/128	123/131	154/156	214/224
899	Hanwell	44	30/06/2001	223/223	257/259	126/126	127/131	154/160	216/218
900	Hanwell	44	30/06/2001	225/225	251/257	128/134	133/139	/	216/224
901	Tolworth	45	03/07/2001	221/225	249/257	126/126	131/137	150/152	216/222
902	Tolworth	45	03/07/2001	219/223	257/257	126/126	129/129	150/162	226/230
903	Tolworth	45	03/07/2001	221/229	241/249	126/128	131/139	146/160	216/226
904	Tolworth	45	03/07/2001	221/221	255/257	126/128	133/139	152/154	216/224
905	Tolworth	45	03/07/2001	231/231	241/255	/	129/137	150/160	214/216
906	Tolworth	45	03/07/2001	221/223	249/251	130/132	123/131	146/152	216/238
907	Tolworth	45	03/07/2001	223/227	255/255	/	129/129	152/154	220/228
908	Tolworth	45	03/07/2001	221/227	249/257	126/134	129/137	146/152	220/224
909	Tolworth	45	03/07/2001	225/225	253/255	/	127/129	152/154	214/216
910	Tolworth	45	03/07/2001	225/225	255/261	126/128	137/147	152/152	214/214
911	St. James Lane	46	23/07/2001	215/221	255/259	126/126	123/131	152/160	/
912	St. James Lane	46	23/07/2001	221/221	253/255	126/126	129/139	158/160	/
913	St. James Lane	46	23/07/2001	221/225	/	126/132	131/133	152/160	222/230
914	St. James Lane	46	23/07/2001	221/227	241/251	124/126	135/137	146/152	214/216
915	St. James Lane	46	23/07/2001	221/225	249/255	130/132	129/133	152/152	/
916	St. James Lane	46	23/07/2001	223/225	255/257	126/130	131/137	154/156	218/230
917	St. James Lane	46	23/07/2001	221/221	253/263	126/126	127/131	146/154	216/220
918	St. James Lane	46	23/07/2001	223/229	253/255	126/126	129/129	150/152	222/230
919	St. James Lane	46	23/07/2001	223/225	257/257	128/130	131/137	146/146	218/230
920	St. James Lane	46	23/07/2001	221/227	241/251	124/126	135/137	146/152	214/214

Appendix F

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Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators

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Abstract

Conservation biologists, evolutionary ecologists and agricultural biologists require an improved understanding of how pollinators utilize space and share resources. Using microsatellite markers, we conducted a genetic analysis of space use and resource sharing at several spatial scales among workers of two ecologically dissimilar bumble bee species (*Bombus terrestris* and *B. pascuorum*) foraging in an urban landscape (London, UK). At fine scales, the relatedness of workers visiting small patches of flowers did not differ significantly from zero. Therefore, colonies shared flower patches randomly with other colonies, suggesting that worker scent-marks deterring visits to unrewarding flowers have not evolved as signals benefiting nestmates. To investigate space use at intermediate scales, we developed a program based on Thomas & Hill's maximum likelihood sibship reconstruction method to estimate the number of colonies utilizing single sites. The average number of colonies (95% confidence limits) sending workers to forage at sites of ≈ 1 ha in area was 96 colonies (84–118) in *B. terrestris* and 66 colonies (61–76) in *B. pascuorum*. These values are surprisingly high and suggested that workers travelled far from their colonies to visit the sites. At the landscape scale, there was little or no genetic differentiation between sites. We conclude that urban habitats support large bumble bee populations and are potentially valuable in terms of bumble bee conservation. In addition, bumble bee-mediated gene flow in plants is likely to occur over large distances and plant–bumble bee conservation requires landscape-scale action.

Keywords: *Bombus*, foraging ecology, microsatellite, plant–pollinator relationship, relatedness, social insect

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Introduction

Knowledge of how pollinators use space and share resources at different spatial scales is required for effective plant–pollinator conservation (Kearns *et al.* 1998; Schulke & Waser 2001; Gathmann & Tscharntke 2002; Steffan-Dewenter *et al.* 2002; Steffan-Dewenter & Kuhn 2003; Dick *et al.* 2003). It is also important for determining patterns of gene flow among pollinated plants (Proctor *et al.* 1996; Cresswell 1997; Schulke & Waser 2001; Cresswell *et al.* 2002), including genetically modified crops (Rieger *et al.* 2002), and the evolutionary basis of communication of resource quality and location among pollinators (Goulson *et al.* 1998, 2000; Stout *et al.* 1998; Williams 1998; Dornhaus & Chittka 1999). However, very little is known about large-scale spatial foraging patterns or resource sharing at any scale in insect pollinators, including bumble bees (Osborne *et al.* 1999; Schulke & Waser 2001; Steffan-Dewenter *et al.* 2002). Bumble bee species are targets for conservation because many wild flowers and commercial crops largely depend on them for pollination and because several bumble bee species are undergoing severe declines (Williams 1982; Matheson *et al.* 1996; Kearns *et al.* 1998). Urban habitats are potentially important for bumble bee conservation because of the presence of flower-rich gardens and parks (Matheson *et al.* 1996; Bernton 2000; Goulson *et al.* 2002). Urban habitats mimic many agricultural landscapes in the fragmented distribution of their resources (Samways 1994).

Bombus terrestris and *B. pascuorum* are common European bumblebees, but *B. terrestris* has large colonies and short-tongued workers that visit a general range of flowers, whereas *B. pascuorum* has smaller colonies and long-tongued workers that specialize on visiting flowers with deep corollae

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(Alford 1975; Prys-Jones & Corbet 1991). Workers of both species deposit scent-marks on flowers that temporarily deter revisitation by themselves, conspecific workers and workers of other bumble bee species (Goulson *et al.* 1998; Stout *et al.* 1998; Williams 1998). Receivers of these signals benefit by avoiding depleted flowers, yet kin selection theory (Hamilton 1964) predicts that selection should not favour the production of costly signals of resource quality benefiting unrelated conspecifics or members of other species. It has, therefore, been suggested, but not demonstrated, that these signals have evolved to benefit either the scent-marking forager herself or her nestmates (Stout *et al.* 1998; Williams 1998).

Studies of foraging bumble bees involving mark–recapture or tracking using harmonic radar have shown that individual workers and colonies tend to be area-constant (favour repeatedly foraging in the same area over periods of hours or days) (Heinrich 1976; Dramstad 1996; Thomson 1996; Saville *et al.* 1997; Osborne *et al.* 1999; Walther-Hellwig & Frankl 2000; Osborne & Williams 2001). They have also shown that workers may fly, on average, hundreds of metres from their nests to forage and some workers may fly 1–2 km (e.g. mean flight distances of 663 m and 275 m, and maxima of 1750 m and 631 m, respectively, in two studies of *B. terrestris*, Osborne *et al.* 1999; Walther-Hellwig & Frankl 2000; see also Dramstad 1996 and Dramstad *et al.* 2003). However, because they are labour-intensive, mark–recapture and radar-tracking measurements of foraging distance have been collected from relatively few colonies. They are also likely to be truncated because, in mark–recapture (Walther-Hellwig & Frankl 2000), observers' search effort falls with increasing distance from the nest, and, in radar-tracking (Osborne *et al.* 1999), workers become undetectable behind obstacles or beyond 700 m. By contrast, genetic analyses permit unbiased, population-wide inferences about average patterns of space use by many colonies simultaneously. They also allow censusing of the number of colonies visiting individual sites.

To characterize space use and resource sharing by bumble bees foraging in an urban landscape, we analysed variation at up to six polymorphic microsatellite DNA loci (Estoup *et al.* 1995, 1996) in 531 *B. terrestris* and 458 *B. pascuorum* workers collected at flowers in 11 sites in London, UK. We conducted our investigation at three spatial scales. First, at a fine scale, we tested the hypothesis that workers visiting the same flower patch are nestmates. Second, at an intermediate scale, we estimated from the genetic data the numbers of colonies sending foraging workers to single sites and the foraging ranges of these workers. Third, at the landscape scale, we analysed worker mixing across sites by estimating between-site genetic differentiation. Darvill *et al.* (submitted) independently investigated space use and resource sharing in *B. terrestris* and *B. pascuorum* at a rural site in Hampshire, UK. Our study and that of Darvill *et al.* are novel because they provide the first genetic estimates of the numbers of colonies visiting sites and of workers' foraging range. Two previous genetic studies estimated the number of colonies contributing to male mating aggregations in social insects (Baudry *et al.* 1998; Paxton 2000), but to our knowledge only the current study and that of Darvill *et al.* (submitted) have estimated the number of colonies represented in a sample of foraging workers. Studies of other invertebrates have also used genetic markers as the basis of indirect census methods (e.g. to estimate the number of foundresses in oak gall-wasps: Atkinson *et al.* 2002). Our work differs from and is complementary to that of Darvill *et al.* (submitted) in that it estimates colony number using a maximum likelihood method, includes an investigation of fine-scale resource sharing, and samples an urban rather than an agricultural environment.

Materials and methods

Field collection and sampling

Between 21 June and 27 July 2001, we collected 74–100 workers off each species at five (*Bombus terrestris*) or four (*B. pascuorum*) sites from sampling areas of mean 0.8 ha (Table 1). The mean distance between sites (Table 1) was 14.7 km (range, 5–27 km). Within each site, up to 10 conspecific workers arriving to forage at each of 10 patches of flowers (mean ± SD area = 0.76 ± 0.25 m², mean distance apart = 57.4 m) were collected in order of their arrival (mean per patch collection times for *B. terrestris* and *B. pascuorum* were 41 and 31 min, respectively). Henceforth, we refer to the collecting sites (Table 1) as 'sites' and to individual patches of flowers as 'patches'. Data from 10 workers collected at each of an additional six sites (Table 1) were used only to estimate between-site genetic differentiation. All collections took place between 09.45 and 18.30. *B. terrestris* workers were distinguished from similar *B. lucorum* workers by a buff 'tail' or a distinct buff line between the 'tail' and the neighbouring black abdominal band (Prys-Jones & Corbet 1991; Benton 2000). Each worker was caught in a plastic tube, chilled and later frozen at −80 °C.

Molecular genetic methods

Workers were genotyped at a mean 5.8 microsatellite loci (range, 3–6) at the loci *B10, B11, B96, B100, B124* and *B126* (*B. terrestris*) and *B96, B118, B124, B126, B131* and *B132* (*B. pascuorum*) (Estoup *et al.* 1995, 1996). DNA was extracted from an entire middle leg using proteinase K digestion in 1× TE buffer (500 µl TE [10 mM Tris–Cl, pH 7.4 and 10 mM EDTA] + 10 µl of 20 mg/mL proteinase K) at 55 °C overnight and then heated to 99 °C for 10 min to

Table 1 Collection details and estimated number of bumble bee colonies visiting sites in London, UK. Flowers making up sampling patches were the following: for *Bombus terrestris, Ballota nigra, Campanula* sp., *Centaurea nigra, Cirsium arvense, Deutzia* sp., *Epilobium* sp., *Galega officinalis, Geranium pratense, Hebe* sp., *Lavendula* sp., *Lotus corniculatus, Rubus fruticosus, Solanum dulcamara, Trifolium repens, T. pratense*; for *Bombus pascuorum, B. nigra, C. nigra, G. officinalis, Lamium album, Lathyrus pratensis, L. corniculatus, R. fruticosus, S. dulcamara, S. nigrum, T. repens, T. pratense, Vicia cracca.* Numbers of colonies are given without correction for unsampled colonies, from sites where 74–100 workers were collected

				B. terrestris		*B. pascuorum*	
Site (postal code)	Grid reference	Habitat type	Sampling area (ha)	No. of workers	No. of colonies	No. of workers	No. of colonies
Nunhead Cemetery SE15	TQ355755	Cemetery	0.85	100	58	100	55
Barnes Common SW13	TQ225759	Public park	0.91	99	66	100	60
Regent's Park NW1	TQ277833	Public park	0.66	74	30	100	53
Millennium Village SE10	TQ399792	Public park	0.70	100	61	98	40
Thames Barrier Park E16	TQ412798	Public park	0.88	98	69	0	n/a
Tolworth Roundabout KT9	TQ198650	Wasteground	0.30	10	n/a	10	n/a
Hanwell Cemetery W7	TQ159800	Cemetery	0.28	10	n/a	10	n/a
Woodgrange Park E12	TQ418851	Cemetery	0.18	10	n/a	10	n/a
St James Lane N10	TQ288895	Garden	0.14	10	n/a	10	n/a
Beddington Park CR0	TQ290654	Public park	0.28	10	n/a	10	n/a
Grove Park SE9	TQ415725	Public park	0.14	10	n/a	10	n/a
Totals				531		458	

n/a, not applicable.

denature the proteinase K prior to polymerase chain reaction (PCR). PCR amplifications were performed using standard protocols (Morin *et al.* 1998). Amplification products were visualized on an ABI PRISM™ 373 automated sequencer and allele sizes were scored using an internal size standard (GeneScanTAMRA 500, Applied Biosystems). The mean numbers of alleles per locus and mean heterozygosities were, respectively, 14.5 (range, 10–20) and 65.5% for *B. terrestris* and 12.5 (range, 9–16) and 67% for *B. pascuorum.*

Across both species, 734 of a total of 5711 genotypes were retyped (using repeat PCRs), from which frequencies of erroneous genotypes were calculated as 4.6% (*B. terrestris* heterozygotes), 23.1% (*B. terrestris* homozygotes), 0% (*B. pascuorum* heterozygotes) and 6.2% (*B. pascuorum* homozygotes). Errors among *B. terrestris* heterozygotes were unlikely to have affected the results because *B. pascuorum*, with no such errors, yielded very similar findings. Most errors among *B. terrestris* homozygotes were due to allelic drop-out (87% involved apparent homozygotes that retyping showed to be heterozygotes). These were also unlikely to have biased the results. First, only a minority (34.5%) of *B. terrestris* genotypes were homozygous, so the overall contribution to the error rate from erroneous homozygotes was 8.0%. Second, relatedness, F_{ST} and colony number estimates from the site accounting for most (56%) of these errors (Nunhead Cemetery) were qualitatively identical to those from a site with no such errors (Regent's Park). Third, simulations (see subsection, 'Estimation of number of colonies utilizing single sites') showed that these errors caused colony number to be underestimated from our datasets and hence to be conservative.

Estimation of linkage disequilibrium and inbreeding

Tests for linkage disequilibrium and for the presence of inbreeding ($F_{IS} > 0$) were carried out on subsamples of workers from each of the sites from which more than 10 workers were collected using GENEPOP 3.1b (Raymond & Rousset 1995), available at http://wbiomed.curtin.edu.au/genepop/. Twenty workers were randomly selected 10 times from each site-sample. This subsampling procedure was designed to minimize the inclusion in the comparisons of nonindependent genotypes due to the presence of relatives in the site-samples. Bonferonni correction was applied for multiple tests.

Estimation of worker relatedness within patches

Because *B. terrestris* and *B. pascuorum* colonies are headed by a single, once-mated queen (Prys-Jones & Corbet 1991; Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000), nestmate workers are full sisters with an expected relatedness (for outbred haplodiploids) of 0.75. Hence the expected relatedness of workers within patches is 0 if workers within sites are randomly distributed over patches, but is 0.75 if single colonies monopolize patches. We therefore calculated regression relatedness (Queller & Goodnight

1989) within patches using RELATEDNESS 5.0.8 (http://gsoft.smu.edu/Gsoft.html) and tested for differences from 0 and 0.75 using *t*-tests. Bonferonni correction was again applied for multiple tests.

Estimation of number of colonies utilizing single sites

Given that workers from the same *B. terrestris* or *B. pascuorum* colony share their mother and father (Prys-Jones & Corbet 1991; Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000), the minimum number of full sisterhoods present in a sample of workers equals the minimum number of colonies represented in the sample. Using allele-sharing criteria for haplodiploid full sisters, we developed a program (COLONY 1.0, available from the authors) adapting the maximum likelihood sibship reconstruction method of Thomas & Hill (2000) to reconstruct worker sibships from each site at which > 10 workers were collected (Table 1). To check the method's power in datasets resembling ours, we simulated the case of a sample of 100 individuals typed at 6 loci each having 10 co-dominant alleles following a uniform frequency distribution, assuming allelic drop-out rates of (i) 0% and (ii) 20% per locus. For (i), when the 'actual' colony size followed a truncated Poisson distribution with $m = 0.9$ and $m = 15$ (where m is the mean size of the workforce representing each colony in the sample), the estimated numbers of colonies were 0.92 ± 0.03 and 1.0 ± 0.0 (mean $\pm$ SD, $n = 100$ replicates) of their actual values, respectively. Therefore, the method's power increased with increasing m, but, for the case of $m = 0.9$, which mimics our data (with many colonies represented in each site sample by a few workers each; see Results), it slightly underestimates colony number. This is because the number of unrelated individuals that, by chance, have multilocus genotypes consistent with full sisterhood increases as colony number increases. For (ii) with $m = 0.9$, the method returned a more conservative estimate (0.85 ± 0.04 of the actual colony number, $n = 100$ replicates) than in (i). Therefore, observed levels of allelic drop-out in our datasets (see 'Molecular genetic methods') caused an additional underestimate of colony number. For larger families ($m >> 0.9$), allelic drop-outs resulted in overestimates of colony number because larger families were split. Effects of other types of error in the genetic data (e.g. scoring or data entry errors) were not investigated. Although such errors might have been present in the data, they were likely to have been rare because, of 139 genotypes scored from 24 additional workers collected from known nests (two *B. terrestris* nests and one *B. pascuorum* nest collected in London; data not shown), only 2 were inconsistent with monogyny and monandry and hence likely to have been scoring or data entry errors. When calculating the average number of *B. terrestris* colonies visiting sites, we excluded the Regent's Park site to equalize the sample sizes (98–100 workers) of included sites (Table 1).

Estimation of foraging distance

The density at which bumble bee nests occur naturally in any environment is unknown at the landscape scale. For scarcer species in nonurban landscapes, the per species density of mature nests successfully producing female sexuals has been estimated at ≈ 1–2 per km² (M. Edwards, pers. commun.). We assumed a range of nest densities of 2–40 nests per km², given that urban bumble bees are likely to occur at relatively high densities (Goulson *et al.* 2002) and not all nests produce female sexuals. This range also approximates the range (2–7 nests per ha per species) measured in two site-scale studies of nest density (Cumber 1953; Harder 1986), correcting for the fact that areas with many nests were selected for investigation in these studies (Cumber 1953; Harder 1986) and the likelihood that such areas are relatively rare across landscapes (Matheson *et al.* 1996). If nests are distributed at density d randomly with respect to foraging sites, the radius r of a circle centred on a site (considered as a point) and enclosing K colonies is $\sqrt{(K/\pi d)}$. Because 50% of colonies will occur in the annulus whose outer and inner borders are at radii r and $(\sqrt{0.5})r$, respectively, from the centre of the circle, median foraging distance was estimated as $(\sqrt{0.5})[\sqrt{(K/\pi d)}]$.

Estimation of between-site genetic differentiation

We estimated levels of between-site genetic differentiation (F_{ST}) using FSTAT (http://www.unil.ch/izea/softwares/fstat.html). To achieve balanced sampling and minimize the inclusion of related workers, we included in the analysis all 10 workers per site from the 6 sites in which only 10 workers were sampled and 10 randomly selected workers per site from the remaining sites. Bonferonni correction was again applied for multiple tests. To investigate the relationship of genetic differentiation and geographical distance, we regressed pairwise F_{ST} on geographical distance, testing the significance of the relationship using a Mantel test with 20 000 permutations.

Results

Linkage disequilibrium and inbreeding

There was no evidence for significant linkage disequilibrium between loci in either species ($n = 10 \times 15$ possible pairwise comparisons between each of the six loci in each species; all $P > 0.0003$, corresponding to table-wide $P = 0.05$). There was also no evidence for significant inbreeding in any site [*Bombus terrestris*: global $F_{IS} = 0.062$ (range, –0.025 to 0.115), $n = 5$ sites, all $P > 0.14$; *B. pascuorum*: global

Table 2 Inbreeding coefficients (F_{IS}) calculated from bumble bee worker samples at sites in London, UK (Table 1), from which 74–100 workers were collected, tested for significant difference from zero. n/a, not applicable

	Bombus terrestris			*Bombus pascuorum*		
Site	F_{IS}	Range	Mean *P*-value	F_{IS}	Range	Mean *P*-value
Nunhead Cemetery	0.1144	0.0432 to 0.2428	0.2560	0.0459	–0.0422 to 0.1145	0.4089
Barnes Common	0.1154	0.0357 to 0.1887	0.2552	0.0744	0.0158 to 0.1728	0.2761
Regent's Park	–0.0251	–0.1808 to 0.0272	0.1436	0.0669	0.0025 to 0.1380	0.2971
Millennium Village	0.1001	0.0443 to 0.2090	0.2163	–0.0028	–0.0972 to 0.0755	0.2823
Thames Barrier Park	0.1093	0.0425 to 0.1948	0.2621	n/a	n/a	n/a

F_{IS} = 0.052 (range, –0.003 to 0.074), n = 4 sites, all $P > 0.28$; Table 2].

Worker relatedness within patches

Mean relatedness of workers visiting patches was 0.008 in *B. terrestris* (range = –0.065 to 0.216, n = 50 patches with mean 9.4 workers each from 5 sites) and 0.012 in *B. pascuorum* (range = –0.068 to 0.147, n = 40 patches with mean 9.9 workers each from 4 sites). In both species, within-patch relatedness was never significantly greater than 0 (*B. terrestris*: all $P > 0.001$, corresponding to table-wide $P = 0.05$; *B. pascuorum*: all $P > 0.0012$, corresponding to table-wide $P = 0.05$) and was always significantly less than 0.75 (*B. terrestris*: all $P < 0.001$; *B. pascuorum*: all $P < 0.0012$).

Number of colonies utilizing single sites

The average minimum number of colonies visiting sites was estimated at 63 colonies per site in *B. terrestris* (n = 4 sites with 99.2 workers genotyped per site) and 52 colonies per site in *B. pascuorum* (n = 4 sites with 99.5 workers genotyped per site) (Table 1). These values are conservative because many colonies visiting sites may have remained unsampled and because our method tended to underestimate colony number from our datasets (see 'Estimation of number of colonies utilizing single sites'). To investigate the scale of the former effect, we pooled datasets from all sites within each species. The observed frequency distributions of the sizes of the workforces from different colonies present in the entire sample did not differ significantly from a truncated Poisson distribution (*B. terrestris*: $\chi^2 = 6.0$, df = 3; *B. pascuorum*, $\chi^2 = 10.4$, df = 5; both $P > 0.05$). We therefore used fitted Poisson distributions to estimate the frequency of colonies unrepresented in the sample and hence the average overall numbers of colonies visiting a site. The resulting overall averages (95% confidence limits) were 96 colonies (84–118) per site for *B. terrestris* and 66 colonies (61–76) per site for *B. pascuorum*.

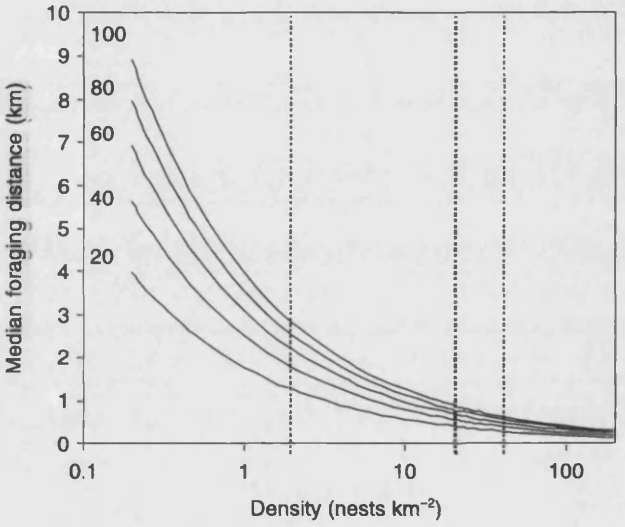

Fig. 1 Estimated median foraging distance as a function of nest density for workers visiting a site attracting K = 20–100 colonies; dotted vertical lines show nest densities of 2, 20 and 40 nests per km^2.

Foraging distance

We combined our estimates of average colony number visiting sites (96 for *B. terrestris* and 66 for *B. pascuorum*) with estimates of nest density (see 'Estimation of foraging distance') to estimate the foraging distances of workers visiting the sites. We estimated median foraging distances to be 0.62–2.8 km for *B. terrestris* and 0.51–2.3 km for *B. pascuorum* (Fig. 1). The corresponding maximum foraging distances were 0.87–3.9 km for *B. terrestris* and 0.72–3.2 km for *B. pascuorum* (Fig. 1).

Between-site genetic differentiation

There was no significant genetic differentiation among sites in *B. terrestris* and slight but significant differentiation in *B. pascuorum* (global F_{ST} [95% confidence limits] = 0.000

[–0.012 to 0.012] and 0.009 [0.003–0.015], respectively). In *B. terrestris*, pairwise differentiation was not significant between any sites (pairwise F_{ST} = –0.0003 to 0.046, n = 55 comparisons between 11 sites, all $P > 0.0009$, corresponding to table-wide $P = 0.05$). In *B. pascuorum*, there was significant pairwise differentiation between only a single pair of sites (pairwise F_{ST} = –0.007 to 0.039, n = 45 comparisons of 10 sites, significant $P = 0.001$). There was no significant correlation between the degree of genetic differentiation among sites and their geographical distance apart in either species (Mantel tests: *B. terrestris*, $r^2 = 6.52\%$; *B. pascuorum*, $r^2 = 0.1\%$; both $P > 0.05$).

Discussion

We conducted a genetic analysis of space use and resource sharing by workers of two bumble bee species (*Bombus terrestris* and *B. pascuorum*) foraging in an urban environment (London, UK). We found that individual colonies did not monopolize flower patches but, instead, workers from different colonies mixed at random within patches. In addition, we found that, surprisingly, many colonies visit, and share forage resources within, individual sites. Hence it is likely that workers foraged far from their nests. Finally, we found no or little genetic differentiation across sites at the landscape scale. Our findings regarding genetic differentiation suggest extensive recent or current gene flow among bee populations across sites, implying that either foraging workers fly far and hence mix across sites, or queens disperse far prior to colony foundation (Mikkola 1984; Stenström & Bergman 1998), or both. They are also consistent with weak or absent genetic differentiation reported in *B. terrestris* and *B. pascuorum* at regional scales (Estoup *et al.* 1996; Widmer & Schmid-Hempel 1999). Our finding that no significant inbreeding was detectable in the study samples is also consistent with previous genetic studies of wild populations of these and other bumble bee species (Owen & Plowright 1980; Estoup *et al.* 1996; Widmer & Schmid-Hempel 1999). Overall, our analyses demonstrate that workers from many colonies mix extensively at the scale of both patches and sites, and suggest that bumble bee workers routinely fly far from their nests (hundreds of metres to several kilometres) to forage. These findings have several implications.

The first implication stems from the finding that, although they are area-constant (Thomson 1996; Saville *et al.* 1997; Osborne *et al.* 1999; Osborne & Williams 2001), individual foragers and colonies clearly share flower patches with unrelated workers from other colonies. This is consistent with lack of recruitment of nestmates to specific locations in bumble bees (Dornhaus & Chittka 1999) and implies that repellent scent-marks left by workers on flowers (Goulson *et al.* 1998; Stout *et al.* 1998; Williams 1998), if they are costly to produce, have not evolved to benefit nestmates, as nestmates would not benefit preferentially from them. They are therefore most likely to have evolved to benefit the individual depositing them (by reducing the chances of its accidentally revisiting an unrewarding flower), or to be nonadaptive (Goulson *et al.* 1998; Stout *et al.* 1998; Williams 1998).

A second implication of our findings is that, although our estimates of foraging range are reliant on uncertain estimates of colony density, bumble bee workers forage even further than previous estimates using mark–recapture or radar-tracking suggested (Osborne *et al.* 1999; Walther-Hellwig & Frankl 2000). Our estimates were also larger than those measured (by translocation experiments) for a range of nonsocial bee species (maxima of 150–600 m: Gathmann & Tscharntke 2002), similar to those deduced (by decoding workers' waggle dances) for honey bees, *Apis mellifera*, foraging over agricultural and wooded landscapes (median foraging distance of 1.2 km: Steffan-Dewenter & Kuhn 2003), but smaller than those deduced (also by decoding workers' waggle dances) for honey bees foraging over moorland (median foraging distance of 6.1 km: Beekman & Ratnieks 2000). Nonetheless, our results suggest that plant gene flow via pollen borne by bumble bee workers is likely to occur over large distances (up to several kilometres), and that flower patches several kilometres from bumble bee nesting areas are likely to receive visits by foraging workers. This knowledge should inform decisions concerning both the conservation of fragmented populations of endangered wild flowers pollinated by bumble bees (Schulke & Waser 2001) and the location of stands of genetically modified crops (Rieger *et al.* 2002). Moreover, if viable pollen is exchanged between workers within the nest as in honey bees (Free & Williams 1972; DeGrandi-Hoffman *et al.* 1986), pollen could be carried by bumble bee workers over distances that are even greater than an individual worker's maximum foraging distance. Extensive mixing of bumble bees from different colonies at flower patches and sites also implies that the potential for horizontal transmission of bumble bee parasites, which are known to be numerous and to have important effects on their hosts' life history (Schmid-Hempel 1998), is large.

The significant difference between the average numbers of colonies visiting single sites in *B. terrestris* and *B. pascuorum*, and hence the difference in their estimated foraging distances, support the suggestion that *B. pascuorum* and species sharing its ecological traits are more localized foragers than *B. terrestris* and similar species (Hedtke & Schricker 1996; Walther-Hellwig & Frankl 2000). Darvill *et al.* (submitted) likewise found that *B. terrestris* had a greater foraging range than *B. pascuorum* and so reached the same conclusion. Note that our comparison of the foraging ranges of *B. terrestris* and *B. pascuorum* assumes similar nest densities in these two species: if

B. pascuorum nests occurred at lower densities, then foraging distances similar to those of *B. terrestris* would also result in fewer *B. pascuorum* colonies being represented at single forage sites, as observed (Table 1; Fig. 1). However, Darvill *et al.* found *B. pascuorum* nests to occur at higher densities than those of *B. terrestris* in an agricultural landscape.

Finally, although for ease of collecting we initially chose sampling sites for the large numbers of workers they attracted, it is clear that resource-rich forage sites in urban areas can serve populations of many colonies from a large surrounding area. This confirms the potential importance of urban sites for bumble bee conservation (Benton 2000). This conclusion is reinforced by the finding of Darvill *et al.* (submitted) that fewer colonies of either species visited sites of similar size in an agricultural landscape, suggesting that these bumble bee species occur at higher density in urban and suburban habitats than in agricultural habitats. Colonies visiting sites in our study were also likely to be utilizing multiple neighbouring forage sites. For all these reasons we conclude that, across all types of environment, conservation strategies for bumble bees and wild flowers dependent on them for pollination should involve coordinated action at a scale larger than that of single sites, namely a landscape scale.

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This work formed part of Roselle Chapman's PhD research, supervised by Andrew Bourke, on the conservation and foraging ecology of urban bumble bees. Andrew Bourke's research focuses on the behavioural ecology, genetics and conservation biology of social insects, especially ants and bumble bees. Jinliang Wang wrote the COLONY program and is interested in developing population and quantitative genetics models and methods of analysis of empirical data to address issues in evolutionary and conservation biology.
